FYI-0902-01424

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July 25, 2002

MR 62337

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Stephen L. Johnson
Assistant Administrator
Office of Prevention, Pesticides, and Toxic Substances TS-7101 OPPTS
Environmental Protection Agency - EAST
7101M
~~Ariel Rose Building~~
1200 Pennsylvania Avenue N
Washington, DC 20460

FYI-02-001424

Dear Mr Johnson:

The American Chemistry Council (Council) makes available to the public and appropriate government agencies final reports of environmental, health, and safety research that it manages. In keeping with this policy, the following two final reports that the Council's Brominated Flame Retardant Industry Panel (BFRIP) recently conducted are enclosed:

- Tetrabromobisphenol A (TBBPA): An Activated Sludge, Respiration Inhibition Test;
- Tetrabromobisphenol A (TBBPA): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants.

In addition, a copy of the report, "Hexabromocyclododecane (HBCD): A 90-Day Oral (Gavage) Toxicity Study of HBCD in Rats, completed December 14, 2000," was sent to your office on January 3, 2002. For completeness a copy of the recently completed addendum to that report is also enclosed.

These reports do not include confidential information.

If you have any questions, please contact Wendy K. Sherman, the BFRIP Manager, at 703/741-5639 or via email at wendy.sherman@americanchemistry.com.

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Sincerely yours,

Susan A. Lewis

Susan A. Lewis, Ph.D.
Managing Director, CHEMSTAR

Contain NO CBI

Enclosures (3)

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TETRABROMOBISPHENOL-A: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-107A

Organisation for Economic Cooperation and Development
OECD Guideline 209

and

Council of European Communities Directive 67/548/EEC
Annex V, Guideline C.11

AUTHORS:
Edward C. Schaefer
Abul I. Siddiqui

STUDY INITIATION DATE: November 21, 2001

STUDY COMPLETION DATE: March 27, 2002

SUBMITTED TO:

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol-A: An Activated Sludge, Respiration Inhibition Test

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-107A

STUDY COMPLETION: March 27, 2002

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in EPA 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17), and Japan MAFF 59 NohSan, Notification No. 3850, Agricultural Production Bureau, with the following exceptions:

The reference substance, obtained from Aldrich Chemical Company (Milwaukee, WI), was not characterized in compliance with Good Laboratory Practice Standards.

The stability of the reference substance under conditions of storage at the test site was not determined in accordance with Good Laboratory Practice Standards.

The homogeneity and stability of the reference material in the carrier was not determined in accordance with Good Laboratory Practice Standards.

STUDY DIRECTOR:

Edward C. Schaefer
Manager, Biodegradation

27 MARCH 2002
DATE

SPONSOR REPRESENTATIVE:

Ms. Wendy Sherman
American Chemistry Council's
Brominated Flame Retardant Industry Panel

March 25, 2002
DATE

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice as published by the U.S. Environmental Protection Agency in EPA 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17); and Japan MAFF 59 NohSan, Notification No, 3850, Agricultural Production Bureau; Wildlife International, Ltd. Standard Operating Procedures and the study protocol. The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY:	DATE CONDUCTED:	DATE REPORTED TO: STUDY DIRECTOR:	MANAGEMENT
Initial Trial: 439E-107			
Test Substance Preparation And Test Initiation	December 12, 2001	December 12, 2001	March 22, 2002
D. O. Measurements	December 12, 2001	December 12, 2001	December 14, 2001
Definitive Trial: 439E-107A			
Test Substance Preparation	December 19, 2001	December 19, 2001	March 26, 2002
Data & Draft Report	January 14 &18, 2002	January 18, 2002	January 22, 2002
Final Report	March 26, 2002	March 26, 2002	March 27, 2002

Robert N. McGee, B.S.
Quality Assurance Representative

March 27, 2002
DATE

REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol-A: An Activated Sludge, Respiration Inhibition Test

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439E-107A

STUDY DIRECTOR:

Edward C. Schaefer
Manager, Biodegradation

27 MARCH 2002
DATE

MANAGEMENT:

Henry O. Krueger, Ph.D.
Director, Aquatic Toxicology and Non-Target Plants

27 Mar 02
DATE

STUDY INFORMATION

Study Initiation Date:	November 21, 2001
Experimental Start Date:	December 11, 2001
Experimental Termination Date:	December 19, 2001
Study Completion Date:	March 27, 2002

Study Director:	Edward C. Schaefer
Sponsor:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
Sponsor's Representative:	Ms. Wendy Sherman
Study Personnel:	Edward C. Schaefer, B.S., Manager, Biodegradation Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants Abul Siddiqui, B.A., Scientist, Biodegradation

TABLE OF CONTENTS

TABLE OF CONTENTS

- Continued -

TABLE

APPENDICES

ABSTRACT

The effect of the test substance on activated sludge microorganisms was assessed by the Activated Sludge Respiration Inhibition Test Method (OECD Guideline 209). The test contained control, reference and treatment groups. The control group was used to determine the background respiration rate of the sludge and was not dosed with the test or reference substance. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at nominal concentrations of 3, 15 and 50 mg/L. The test substance was dosed at a limit concentration of 15 mg/L. After an exposure period of approximately three hours, the respiration rates of the test solutions were measured using a dissolved oxygen meter. The individual respiration rates of the two controls were 15.3 and 17.1 mg O_2/L/hr. The difference between the two control respiration rates was 10.5% and was within the 15% difference limit established for the test. The validity of the test was further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 9.6 mg/L. The EC50 was within the 5 to 30 mg/L range considered acceptable for the test. An average of approximately 1.9 percent inhibition was observed in the treatment group. Following is a summary of the results:

Treatment/Nominal Concentration	Respiration Rate mg O_2/L/hour	Percent Inhibition
Control 1	15.3	NA
Control 2	17.1	NA
3,5-dichlorophenol 3 mg/L	15.3	5.9
3,5-dichlorophenol 15 mg/L	4.9	69.9
3,5-dichlorophenol 50 mg/L	2.4	85.2
Tetrabromobisphenol-A 15 mg/L	15.9	1.9
Tetrabromobisphenol-A 15 mg/L	15.9	1.9
Tetrabromobisphenol-A 15 mg/L	15.9	1.9

NA – Not applicable

INTRODUCTION

The purpose of this test is to provide a screening method to identify substances that may adversely affect aerobic microbial treatment plants and to indicate suitable non-inhibitory test substance concentrations for use in biodegradability tests.

This study was conducted by Wildlife International, Ltd. for the American Chemistry Council's Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. biodegradation facility in Easton, Maryland. Original raw data generated by Wildlife International, Ltd. and the original final report are filed under Project Number 439E-107A in the archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to assess the effects of tetrabromobisphenol-A oxide on activated sludge microorganisms by measuring the respiration rate.

EXPERIMENTAL DESIGN

The test contained control, reference, and treatment groups. The control group was used to determine the background respiration rate of the sludge and was not exposed to the test or reference substances. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at nominal concentrations of 3, 15 and 50 mg/L. The test substance was tested at a limit concentration of 15 mg/L, in triplicate.

MATERIALS AND METHODS

This study was conducted according to the procedures outlined in the protocol, "Tetrabromobisphenol A: An Activated Sludge, Respiration Inhibition Test," (Appendix II). The protocol was based on the procedures specified in the OECD Guideline for Testing of Chemicals, Method 209 (1) and Council of the European Communities, Guideline C.11, Activated Sludge, Respiration Inhibition Test (2).

Test Substance

The test substance used in this study was a composite of the following three samples:

Manufacturer:	Bromide Compounds Ltd
Sample ID:	Tetrabromobisphenol-A
Description	Powder
Purity	>99% Tetrabromobisphenol-A
Lot No.:	000135
CAS No:	Not given
Expiration Date:	Not given
Date Received:	August 17, 2000
Wildlife International, Ltd. ID:	5354

Manufacturer:	Great Lakes Chemical Corporation
Sample ID:	Tetrabromobisphenol A
Description	White powder
Purity	Not given
Batch No.:	008JG21C
CAS No:	00079-94-7
Expiration Date:	Not given
Date Received:	July 25, 2000
Wildlife International, Ltd. ID:	5315

Manufacturer:	Albemarle Corporation
Sample ID:	Tetrabromobisphenol-A (TBBPA)
Description	White powder
Purity	Not given
Lot No.:	25938C-1
CAS No:	79-94-7
Expiration Date:	Not given
Date Received:	July 27, 2000
Wildlife International, Ltd. ID:	5318

The composite tetrabromobisphenol-A sample was prepared on September 19, 2000 and was assigned Wildlife International Ltd. identification number 5381. The composite sample was prepared by combining equal parts of the three manufacturers' products and mixing for approximately ten minutes.

The test substance was administered to the treatment group by direct weight addition.

Reference Substance

A stock solution of the reference substance, 3,5-dichlorophenol was prepared by dissolving 500 mg in 10 mL of 1N NaOH and then diluting to 30 mL with NANOpure® water. While stirring, enough 1N H_2SO_4 was added to reach the point of incipient precipitation. The solution of 3,5-dichlorophenol then was diluted to 1 L with NANOpure® water. The reference substance was administered by volumetric addition. Following is a description of the reference substance used in this study.

Name:	3,5-dichlorophenol
Manufacturer:	Aldrich Chemical Co., Milwaukee, WI
Lot Number:	02611ES
Physical Description:	White solid
Handling Precautions:	Standard laboratory precautions
Date Received:	January 24, 2000
Expiration Date:	January 24, 2005
Purity:	99.1%
Storage Conditions:	Ambient
CAS Number:	591-35-5
Wildlife International, Ltd. ID:	5179

Test Conditions and Apparatus

Control, reference, and treatment test mixtures were incubated at 20 ± 2°C and aerated for three hours at a rate sufficient to provide aerobic conditions and maintain solids in suspension. The mixtures were prepared and aerated in 500 mL plastic Erlenmeyer flasks and then transferred into 300 mL biochemical oxygen demand (BOD) bottles to conduct the dissolved oxygen (DO) measurements.

Test Inoculum

Activated sludge was collected from the Denton Wastewater Treatment Plant, Denton, Maryland on December 18, 2001. The Denton facility receives wastes from predominately domestic sources. The sludge was sieved using a 2 mm screen and allowed to settle for approximately 30 minutes. After the settling period, the supernatant was removed and the total suspended solids (TSS) concentration of the settled sludge was determined.

The sludge was maintained in the laboratory for 1 day prior to use. Approximately 50 mL of synthetic sewage (Protocol, Appendix II) was added to each liter of activated sludge and the sludge was continuously aerated. Before use, the pH and total suspended solids concentration of the activated sludge were determined.

Procedure

Test mixtures were prepared at 15 minute intervals starting with the first control. The control contained 9.6 mL of synthetic sewage, 120 mL of inoculum, and enough municipal water to bring the total volume up to 300 mL. The mixture was promptly aerated at a rate sufficient to provide aerobic conditions and keep the solids in suspension. Subsequent mixtures contained 9.6 mL of synthetic sewage, 120 mL of inoculum, the appropriate amount of test substance or reference substance stock solution, and enough municipal water to bring the total volume up to 300 mL. Finally, a second control was prepared. All mixtures were aerated for three hours.

Sample Analysis

After three hours of aeration, the contents of the first vessel were transferred to a BOD bottle and the respiration rate was measured over a period of up to 10 minutes. Dissolved oxygen readings were recorded every 10 seconds for 10 minutes or until the DO dropped below 1.0 mg/L, whichever came first using a YSI Model 50B Dissolved Oxygen Meter. The respiration rate in subsequent vessels was determined in an identical manner at 15 minute intervals so that the contact time of the test substance with the activated sludge was three hours.

Calculations

A respiration rate was calculated for each test mixture and expressed in mg O_2/L/hour. The rate was calculated using DO values between approximately 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10 minute period if the DO did not reach approximately 2.5 mg O_2/L. The respiration rate was calculated using the following equation:

$$\textit{Respiration Rate} = \textit{(initial DO – final DO)/(final time – initial time)}$$

Percent inhibition was calculated using the following equation:

$$\textit{Percent Inhibition} = 1 - \frac{2R_s}{RC_1 + RC_2} \; X\,100$$

where:

R_s = oxygen consumption rate at a given concentration of the test substance
RC_1 = oxygen consumption rate, Control 1
RC_2 = oxygen consumption rate, Control 2

Statistical Analyses

When the dose response pattern allows for the calculation of an EC50 value, the data are analyzed using the computer program of C.E. Stephan (3). The program was designed to calculate the EC50 value and the 95% confidence interval by probit analysis, the moving average, or binomial probability with nonlinear interpolation (4, 5, 6). The EC50 value for the reference group was calculated using nonlinear interpolation.

RESULTS AND DISCUSSION

The temperature range during the maintenance of the sludge and during the test was 20-22° C. The measured total suspended solids (TSS) concentration and pH of the sludge on the day of testing was 3640 mg/L and 7.8, respectively.

Respiration rates and percent inhibitions are presented in Table 1. The respiration rates in the two controls were 15.3 and 17.1 mg O_2/L/hr. The difference between the two control respiration rates was 10.5% and was within the 15% difference limit established for the test. The validity of the test was

further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 9.6 mg/L. The EC50 was within the 5 to 30 mg/L range considered acceptable for the test.

Minimal inhibitory effects upon respiration were observed at a tetrabromobisphenol-A concentration of 15 mg/L. The average respiration rate for the treatment group was 15.9 ± 0.0 O_2/L/hr and was slightly lower than the average respiration rate of the control (16.2 ± 1.3 mg O_2/L/hr). The average percent inhibition observed was approximately 1.9%.

CONCLUSION

Minimal inhibitory effects upon respiration were observed at a tetrabromobisphenol-A concentration of 15 mg/L. The average percent inhibition observed was approximately 1.9%.

REFERENCES

1. **Organisation for Economic Cooperation and Development.** 1989. *Activated Sludge Respiration Inhibition Test.* OECD Guideline 209.

2. **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.11, *Activated Sludge Respiration Inhibition Test.*

3. **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

4. **Finney, D.J.** 1971. *Statistical Methods in Biological Assay,* second edition. Griffin Press, London.

5. **Thompson, W.R.** 1947. *Bacteriological Reviews,* Vol. II, No. 2: 115-145.

6. **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

Table 1

Respiration Rates and Percent Inhibitions

Treatment/Nominal Concentration	Respiration Rate mg O_2/L/hour	Percent Inhibition
Control 1	15.3	NA
Control 2	17.1	NA
3,5-dichlorophenol 3 mg/L	15.3	5.9
3,5-dichlorophenol 15 mg/L	4.9	69.9
3,5-dichlorophenol 50 mg/L	2.4	85.2
Tetrabromobisphenol-A 15 mg/L	15.9	1.9
Tetrabromobisphenol-A 15 mg/L	15.9	1.9
Tetrabromobisphenol-A 15 mg/L	15.9	1.9

NA – Not applicable.

APPENDIX I

Measured Dissolved Oxygen (DO) Concentrations (mg O_2/L)

Time (min./sec.)	Control 1	Reference			Treatment			Control 2
		3 mg/L	15 mg/L	50 mg/L	Rep A 15 mg/L	Rep B 15 mg/L	Rep C 15 mg/L	
00:10	**8.4**	**8.4**	**8.5**	**9.0**	**8.0**	**8.2**	**8.1**	**8.1**
00:20	8.4	8.4	8.7	9.1	8.0	8.2	8.1	8.1
00:30	8.3	8.3	8.7	9.2	8.0	8.2	8.1	8.0
00:40	8.3	8.3	8.6	9.2	7.9	8.1	8.0	8.0
00:50	8.3	8.2	8.6	9.2	7.9	8.1	8.0	7.9
00:60	8.2	8.2	8.6	9.2	7.9	8.0	7.9	7.9
00:70	8.2	8.2	8.6	9.2	7.8	8.0	7.9	7.9
00.80	8.2	8.1	8.6	9.2	7.8	7.9	7.8	7.8
00.90	8.1	8.1	8.6	9.2	7.7	7.9	7.8	7.8
01.00	8.1	8.1	8.6	9.2	7.7	7.8	7.8	7.7
01:10	8.0	8.0	8.6	9.1	7.6	7.8	7.7	7.7
01:20	8.0	8.0	8.6	9.1	7.6	7.8	7.7	7.6
01:30	8.0	7.9	8.5	9.1	7.6	7.7	7.6	7.6
01:40	7.9	7.9	8.5	9.1	7.5	7.7	7.6	7.5
01:50	7.9	7.9	8.5	9.1	7.5	7.6	7.6	7.5
01:60	7.8	7.8	8.5	9.1	7.4	7.6	7.5	7.4
01:70	7.8	7.8	8.5	9.0	7.4	7.5	7.5	7.4
01:80	7.7	7.7	8.5	9.0	7.3	7.5	7.4	7.4
01:90	7.7	7.7	8.5	9.0	7.3	7.4	7.4	7.3
02:00	7.7	7.6	8.4	9.0	7.2	7.4	7.3	7.3
02:10	7.6	7.6	8.4	9.0	7.2	7.4	7.3	7.2
02:20	7.6	7.6	8.4	9.0	7.2	7.3	7.2	7.2
02:30	7.5	7.5	8.4	9.0	7.1	7.3	7.2	7.1
02:40	7.5	7.5	8.4	9.0	7.1	7.2	7.1	7.1
02:50	7.4	7.4	8.3	9.0	7.0	7.2	7.1	7.0
02:60	7.4	7.4	8.3	9.0	7.0	7.1	7.0	7.0
02:70	7.4	7.4	8.3	9.0	6.9	7.1	7.0	6.9
02:80	7.3	7.3	8.3	9.0	6.9	7.0	7.0	6.9
02:90	7.3	7.3	8.3	9.0	6.8	7.0	6.9	6.8
03:00	7.2	7.2	8.2	8.9	6.8	7.0	6.9	6.8
03:10	7.2	7.2	8.2	8.9	6.8	6.9	6.8	6.7
03:20	7.1	7.2	8.2	8.9	6.7	6.9	6.8	6.7
03:30	7.1	7.1	8.2	8.9	6.7	6.8	6.7	6.6
03:40	7.0	7.1	8.2	8.9	6.6	6.8	6.7	6.6
03:50	7.0	7.0	8.2	8.9	6.6	6.7	6.6	6.5
03:60	6.9	7.0	8.1	8.9	6.6	6.7	6.6	6.5
03:70	6.9	6.9	8.1	8.9	6.5	6.6	6.6	6.4
03:80	6.9	6.9	8.1	8.9	6.5	6.6	6.5	6.4
03:90	6.8	6.8	8.1	8.8	6.4	6.6	6.5	6.3
04:00	6.8	6.8	8.0	8.8	6.4	6.5	6.4	6.3
04:10	6.7	6.8	8.0	8.8	6.3	6.5	6.4	6.2
04:20	6.7	6.7	8.0	8.8	6.3	6.4	6.3	6.2
04.30	6.6	6.7	8.0	8.8	6.2	6.4	6.3	6.1
04.40	6.6	6.6	7.9	8.8	6.2	6.3	6.2	6.1
04:50	6.6	6.6	7.9	8.8	6.1	6.3	6.2	6.1
04:60	6.5	6.5	7.9	8.8	6.1	6.2	6.1	6.0
04:70	6.5	6.5	7.9	8.8	6.1	6.2	6.1	6.0
04:80	6.4	6.4	7.9	8.8	6.0	6.1	6.1	5.9
04:90	6.4	6.4	7.9	8.7	6.0	6.1	6.0	5.9
05:00	6.4	6.4	7.9	8.7	5.9	6.1	6.0	5.8
05:10	6.3	6.3	7.8	8.7	5.9	6.0	5.9	5.8
05:20	6.3	6.3	7.8	8.7	5.8	6.0	5.9	5.7
05:30	6.3	6.2	7.8	8.7	5.8	5.9	5.8	5.7
05:40	6.2	6.2	7.8	8.7	5.7	5.9	5.8	5.6
05:50	6.2	6.1	7.8	8.7	5.7	5.8	5.7	5.6
05:60	6.1	6.1	7.8	8.7	5.6	5.8	5.7	5.5
05:70	6.1	6.1	7.8	8.6	5.6	5.7	5.6	5.5
05:80	6.0	6.0	7.7	8.6	5.5	5.7	5.6	5.4
05:90	6.0	6.0	7.7	8.6	5.5	5.6	5.6	5.4
06:00	**5.9**	**5.9**	**7.7**	**8.6**	**5.4**	**5.6**	**5.5**	**5.3**

Bold numbers indicate dissolved oxygen concentrations used to calculate respiration rates.

APPENDIX II

Protocol

PROTOCOL

TETRABROMOBISPHENOL A: AN ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

Organization for Economic Cooperation and Development
OECD Guideline 209

and

Council of European Communities Directive 67/548/EEC
Annex V, Guideline C.11

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

November 7, 2001

Wildlife International, Ltd.

- 2 -

TETRABROMOBISPHENOL A: AN ACTIVATED SLUDGE,
RESPIRATION INHIBITION TEST

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Edward C. Schaefer
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Manager of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 12/04/01 Experimental Termination Date: 12/10/01

Project No.: 439E-107

Test Concentrations: 15 mg/L

Test Substance No.: 5381 Reference Substance No. (if applicable): 5179

PROTOCOL APPROVAL

Edward C. Schaefer
STUDY DIRECTOR — DATE 11/21/01

Willard K. Krueger
LABORATORY MANAGEMENT — DATE 11/21/01

Wendy K. Sherman
SPONSOR'S REPRESENTATIVE — DATE 11/16/01

Wildlife International, Ltd.

INTRODUCTION

The purpose of this test is to provide a screening method to identify substances that may adversely affect aerobic microbial treatment plants and to indicate suitable non-inhibitory test substance concentrations for use in biodegradability tests.

OBJECTIVE

The objective of the study will be to assess the effects of the test substance on activated sludge microorganisms by measuring the respiration rate. An EC50 will be calculated, if possible.

EXPERIMENTAL DESIGN

The test will contain control, reference, and treatment groups. The control group is used to determine the background respiration rate of the sludge and will not be exposed to the test substance. The reference group will be dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at concentrations of 3, 15, and 50 mg/l. The test substance will be tested at a limit concentration of 15 mg/l, in triplicate.

MATERIALS AND METHODS

Test methods are based on the procedures specified in the OECD Guideline for Testing of Chemicals, Method 209 (1) and Council of the European Communities, Guideline C.11, Activated Sludge, Respiration Inhibition Test (2).

Test Substance

The test substance consisted of a composite of TBBPA samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. Identification Number
Great Lakes Chemical Corporation	008JG21C	July 25, 2000	5315
Albemarle Corporation	25938C-1	July 27, 2000	5318
Bromine Compounds, Ltd.	000135	August 17, 2000	5354

The composite test substance was assigned Wildlife International Ltd. identification number 5381 and is being stored under ambient conditions.

Wildlife International, Ltd.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

The test substance will be administered by direct weight addition. Direct weight addition is the most appropriate route of administration of insoluble materials.

Stock Solution Preparation

A stock solution of 3,5-dichlorophenol will be prepared by dissolving 500 mg in 10 mL of 1N NaOH and then diluting to 30 mL with NANO™pure water. While stirring, enough 1N H_2SO_4 (approximately 8 mL) will be added to reach the point of incipient precipitation. The solution of 3,5-dichlorophenol then will be diluted to 1 L with NANO™pure water. The reference substance will be administered by volumetric addition.

Test System

The biological test system is a consortium of microorganisms common to the activated sludge treatment process. The organisms responsible for the decomposition of organic materials are principally aerobic, and facultative bacteria. The test system was chosen because it is representative of a treatment process that may receive the test substance.

Test Conditions and Apparatus

Control, reference, and treatment test mixtures will be incubated at 20 ± 2°C and aerated for 3 hours at a rate sufficient to maintain solids in suspension. The mixtures will be prepared and aerated in 500 mL plastic Erlenmeyer flasks and then transferred into a 300 mL Biochemical Oxygen Demand (BOD) bottle to conduct dissolved oxygen (DO) measurements. Test mixtures will be identified by project number, test substance identification and test concentration.

Test Inoculum

Activated sludge from the Denton Wastewater Treatment Plant, Denton, Maryland will be used as the inoculum for the test. The sludge will be sieved using a 2 mm screen and then allowed to settle for approximately 30 minutes. The supernatant above the settled solids will be drained and the total

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suspended solids (TSS) concentration of the settled sludge will be determined. Based on the result, the concentration of the sludge will be adjusted to 4000 mg/L (± 10%) by diluting with Nanopure® water.

If the sludge cannot be used on the day of collection or if the same batch is required to be used on subsequent days (maximum four days), 50 mL of synthetic sewage (Appendix II) will be added to each liter of activated sludge at the end of each working day. The sludge will be aerated overnight at 20 ± 2°C. Before use, the pH and total suspended solids concentration of the activated sludge will be determined and, if necessary, adjusted to pH 6.0 - 8.0 and a solids concentration of 4000 mg/L (± 10%).

Procedure

Test mixtures will be prepared at 15 minute intervals starting with the first control. The control will contain 9.6 mL of synthetic sewage, 120 mL of inoculum and enough municipal water to bring the total volume up to 300 mL. The mixture will be promptly aerated at a rate sufficient to keep the solids in suspension. Subsequent mixtures will contain 9.6 mL of synthetic sewage, 120 mL of inoculum, the appropriate amount of test or reference substance, and enough municipal water to bring the total volume up to 300 mL. Finally, a second control will be prepared. All mixtures will be aerated for three hours.

Sample Analysis

After three hours of aeration, the contents of the first vessel will be transferred to a BOD bottle and the respiration rate will be measured over a period of up to 10 minutes. Dissolved oxygen readings will be recorded every 10 seconds for 10 minutes or until the DO drops below 1.0 mg/L, which ever occurs first, The respiration rate in subsequent vessels will be determined in an identical manner at 15 minute intervals so that the contact time of the test substance with the activated sludge is three hours.

Calculations

A respiration rate will be calculated for each test mixture and expressed in mg O_2/L/hour. The rate will be calculated using DO values between approximately 6.5 mg O_2/L and 2.5 mg O_2/L, or over a 10 minute period if the DO does not reach approximately 2.5 mg O_2/L. The respiration rate will be calculated as follows:

$$\textit{Respiration Rate} = \textit{(initial DO} - \textit{final DO)/(final time} - \textit{initial time)}$$

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The percent inhibition for each test substance concentration will be calculated using the following equation and plotted against concentration on log paper:

$$Percent\ Inhibition = 1 - \frac{2R_s}{RC_1 + RC_2} X\ 100$$

where

R_s = oxygen consumption rate at a given concentration of the test substance

RC_1 = oxygen consumption rate, Control 1

RC_2 = oxygen consumption rate, Control 2

An EC50 value will be derived, if possible, based on the percent inhibition versus test substance concentration. Confidence limits (95%) for the EC50 will be determined using standard statistical procedures (3).

Quality Control

The test is considered valid only if the following criteria are met:

- the two control respiration rates are within 15% of each other;
- the EC50 (3 hours) of 3,5-dichlorophenol is in the accepted range of 5 to 30 mg/L.

RECORDS TO BE MAINTAINED

Records to be maintained will include, but not limited to, the following:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance as provided by Sponsor.
3. Test initiation and termination dates.
4. Experimental initiation and termination dates.
5. Stock solution concentration calculations and solution preparation.
6. Activated sludge source and pretreatment details.
7. Test temperature and duration.
8. Reference substance results.

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9. All dissolved oxygen measurements.
10. Temperature range recorded during test period.
11. Inhibition curve and method for calculation of EC50.
12. If calculated, EC50 and 95% confidence limits.
13. A copy of the final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report is to include, but is not limited to, the following when applicable:

1. Name and address of facility performing the study.
2. Dates on which the study was initiated and completed.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
5. Identification and characterization of the test substance as provided by Sponsor including name, CAS number, percent active, and other characteristics, if provided by the Sponsor.
6. A description of the transformations and calculations performed on the data.
7. A description of the methods used and reference to any standard method employed.
8. A description of the test system.
9. A description of the preparation of the test solutions, the testing concentration(s), the route of administration, and the duration of the test.
10. A description of all circumstances that may of affected the quality or integrity of the data.
11. The name of the study director, the names of other scientists or professionals, and the names of all supervisory personnel, involved in the study.
12. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
13. The location where the raw data and final report are to be stored.
14. A statement prepared by the Quality Assurance Unit listing the dates that the study inspections and audits were made and the dates of any findings were reported to the Study Director and Management.

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15. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.
16. A copy of the signed protocol and amendments.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160); OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17); and Japan MAFF (59 NohSan, Notification No. 3850, Agricultural Production Bureau). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

Wildlife International, Ltd.

REFERENCES

1 **Organisation for Economic Cooperation and Development.** 1989. *Activated Sludge Respiration Inhibition Test.* OECD Guideline 209.

2 **Council of the European Communities.** Directive 67/548/EEC. Annex V. Guideline C.11, *Activated Sludge Respiration Inhibition Test.*

3 **Stephan, C.E.** 1977. "Methods for Calculating an LC50," *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

Wildlife International, Ltd.

- 10 -

APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): Tetrabromobisphenol-A

Reference Standard (if applicable): N/A

Test Substance Sample Code or Batch Number: Wildlife International, Ltd. No. 5381

Test Substance Purity (% Active Ingredient): 98.91% Expiration Date: August 1, 2002

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? X Yes ___ No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd..

Ambient temperature; protect from light and moisture

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? X Yes ___ No

Other pertinent stability information:

N/A

IV. Toxicity Information:

Mammalian: Rat LD50 > 5 g/kg Mouse LD50: > 10 g/kg

Aquatic:	Invertebrate Toxicity (EC/LC50)	Fish Toxicity (LC50)
	N/A	N/A

Other Toxicity Information (including findings of chronic and subchronic tests):

V. Classification of the Compound:

_____Insecticide _____Herbicide _____Fungicide

_____Microbial Agent _____Economic Poison

Other: Halogenated flame retardant

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APPENDIX II. SYNTHETIC SEWAGE

The synthetic sewage provides the necessary nutrients required for bacterial metabolism. It is prepared by dissolving the following amounts of substances in 1 liter of municipal water:

16.0 g peptone
11.0 g meat extract
3.0 g urea
0.7 g NaCl
0.4 g $CaCl_2$ $2H_2O$
0.2 g $MgSO_4$ $7H_2O$
2.8 g K_2HPO_4

Reagent grade chemicals or better will be used when available. The constituents of the synthetic sewage are not known to contain any contaminants that are reasonable expected to be present and are known to be capable of interfering with the study.

TETRABROMOBISPHENOL A: A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS

FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 439-102

OECD Guideline for Testing of Chemicals
Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests

and

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.4100 and 850.4225

AUTHORS:

John R. Porch
Timothy Z. Kendall
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: November 7, 2001

STUDY COMPLETION DATE: March 5, 2002

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol A: A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439-102

STUDY COMPLETION: March 5, 2002

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17); and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999.

STUDY DIRECTOR:

John R. Porch — 5 March 02

John R. Porch — Date

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM(98)17); and Japan MAFF, 11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999. The dates of all audits and inspections and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY	DATE CONDUCTED	STUDY DIRECTOR	MANAGEMENT
Test Substance Preparation	November 14, 2001	November 14, 2001	November 14, 2001
Initiation	November 14, 2001	November 14, 2001	November 15, 2001
Matrix Fortifications	November 15, 2001	November 15, 2001	November 15, 2001
Observations and Height Measurements	December 5, 2001	December 5, 2001	December 5, 2001
Analytical Data and Draft Report	January 8, 2002	January 8, 2002	January 8, 2002
Biological Data and Draft Report	January 7-10, 2002	January 10, 2002	March 5, 2002
Final Report	March 4, 2002	March 4, 2002	March 5, 2002

Kimberly A. Hoxter
Kimberly A. Hoxter
Quality Assurance Representative

3-5-02
Date

REPORT APPROVAL

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel

TITLE: Tetrabromobisphenol A: A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

WILDLIFE INTERNATIONAL, LTD. PROJECT NO.: 439-102

STUDY DIRECTOR:

John R. Porch — 5 March 02
John R. Porch, Supervisor, Non-Target Plants and Insects — Date

CHEMISTRY PRINCIPAL INVESTIGATOR:

Timothy Z. Kendall — 3/5/02
Timothy Z. Kendall, Supervisor — Date

WILDLIFE INTERNATIONAL, LTD. MANAGEMENT:

Henry O. Krueger — 5 Mar 02
Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants — Date

Willard B. Nixon — 3/5/02
Willard B. Nixon, Ph.D., Director, Analytical Chemistry — Date

TABLE OF CONTENTS

TABLE OF CONTENTS
(continued)

TABLES

APPENDICES

SUMMARY

WILDLIFE INTERNATIONAL, LTD. PROJECT NO: 439-102

TEST SUBSTANCE: Tetrabromobisphenol A

STUDY TITLE: Tetrabromobisphenol A: A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

GUIDELINES: OECD Guideline for Testing of Chemicals, Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests
OPPTS 850.4100 (Public Draft)
OPPTS 850.4225 (Public Draft)

NOMINAL TEST LEVELS: 0 (Control), 20, 78, 313, 1250, 5000 mg/kg dry soil

TEST DATES:

STUDY INITIATION:	November 7, 2001
Experimental Start (OECD):	November 14, 2001
Experimental Start (EPA):	November 14, 2001
Experimental Termination:	December 7, 2001
STUDY COMPLETION:	March 5, 2002

LENGTH OF TEST: 21 days

TEST SPECIES: Corn (*Zea mays*), Cucumber (*Cucumis sativa*), Onion (*Allium cepa*), Ryegrass (*Lolium perenne*), Soybean (*Glycine max*), Tomato (*Lycopersicon esulentum*)

RESULTS: The NOEC, LOEC, and lowest Effect Concentration (EC_x) for each of the six test species is listed below. This summary is based on the most sensitive endpoints only, complete results are presented in the text and tables.

RESULTS[1]:	Corn	Cucumber	Onion	Ryegrass	Soybean	Tomato
Most sensitive endpoint(s)	Dry Weight	Dry Weight, Height	Dry Weight, Height	Dry Weight, Height	-[2]	Dry Weight, Height
NOEC	313	20	313	78	5000	313
LOEC	1250	78	1250	313	>5000	1250
EC_{25}	>5000	73	460	49	>5000	422
EC_{50}	>5000	1672	4264	459	>5000	>5000
LC_{50}	>5000	>5000	>5000	>5000	>5000	>5000

[1] Results are presented as mg Tetrabromobisphenol A/kg soil dry weight.
[2] " –" indicates that endpoint or concentration could not be obtained because there were no effects observed

INTRODUCTION

This seedling emergence study was conducted for American Chemistry Council's Brominated Flame Retardant Industry Panel at the Wildlife International, Ltd. greenhouse facility in Easton, Maryland. The in-life portion of the test was conducted from November 14, 2001 to December 5, 2001. Raw data generated at Wildlife International, Ltd., the study protocol, and a copy of the final report were filed in the archives located on the Wildlife International, Ltd. site. Key personnel involved in the study are listed in Appendix 1.

PURPOSE

The purpose of the study was to determine the effects of Tetrabromobisphenol A (TBBPA) on the seedling emergence and growth of six species of non-target plants.

EXPERIMENTAL DESIGN

The experimental design for this study consisted of a negative control and five treatment groups. Each group had four replicate pots with ten seeds planted in each pot. Test concentrations of TBBPA were made by soil incorporation to each treatment group prior to the planting of seeds. The nominal test substance concentrations were 20, 78, 313, 1250, and 5000 mg of TBBPA per kilogram of dry soil (mg/kg). A control group, which received no test substance incorporation, was maintained concurrently.

Seeds were impartially assigned to prelabelled growth pots on the day of test initiation. The replicate pots were placed in a randomized block design on a greenhouse table after planting. Observations of emergence were made on Days 7, 14, and 21. A general assessment of seedling condition was made on Day 7, while observations of height, shoot dry weight, and assignment of plant condition scores were made only on Day 21.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Tetrabromobisphenol A: A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants" (Appendix 2). The methods used in conducting this study were based upon procedures specified in the OECD Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests (1) and the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Numbers 850.4100 (2) and 850.4225 (3).

Test Substance

The test substance consisted of a composite of Tetrabromobisphenol A samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International, Ltd. Identification Number
Great Lakes Chemical Corporation	1008JE04B	August 16, 2001	5722
Albemarle Corporation	25115T-1	August 16, 2001	5721
Bromine Compounds, Ltd.	010040	August 31, 2001	5733

The composite test substance was assigned Wildlife International, Ltd. identification number 5754 and was stored under ambient conditions. The composite test substance was shipped to Albemarle Corporation for characterization and purity analyses (Appendix 3). The results of the analyses indicated the composite test substance was homogeneous. The conclusion of the characterization was that the test article had a purity of 99.17%.

Preparation and Soil Incorporation of Test Substance

The test soil was prepared by mixing TBBPA into bulk test soil with a measured soil moisture of 15%. Test substance for treatment groups 20, 78, 313, 1250, and 5000 mg/kg was prepared by weighing five known weights (1.0, 4.0, 16.0, 63.8, and 255.0 g) of TBBPA. Approximately 60 kg of bulk soil was measured into a soil mixer, and weighed test substance was added for each test concentration. The test substance and bulk soil were then mixed for ten minutes in order to prepare the test soil for each treatment group. Soils were mixed from lowest to highest concentration to avoid cross-contamination. The negative control soil was prepared in the same manner as the other test groups, but no test substance was added. At the completion of mixing, the test soils were sampled to provide material for analytical confirmation of the test concentrations. Analytical samples were stored at ambient room conditions after their collection until they were analyzed.

Test Species

The common and scientific names for the six species tested, the seed source, and their approximate planting depths are listed below:

Test Species / Variety:	Seed Source:	Planting Depth
Corn (*Zea mays*) / Mandan Bride	Johnny's Selected Seeds, Albion, ME, USA	20 mm
Onion (*Allium cepa*) / Texas Grano	Territorial Seed Co., Cottage Grove, OR, USA	6 mm
Ryegrass (*Lolium perenne*) / Manhattan 3	Meyer Seed Co., Baltimore, MD, USA	6 mm
Cucumber (*Cucumis sativa*) / Straight Eight	Meyer Seed Co., Baltimore, MD, USA	20 mm
Soybean (*Glycine max*) / Green Envy	Johnny's Selected Seeds, Albion, ME, USA	20 mm
Tomato (*Lycopersicon esculentum*) /Rutgers	Meyer Seed Co., Baltimore, MD, USA	6 mm

These species were chosen because they represent ecologically important families, and are readily cultivated test organisms that are widely used in research. Seeds were selected from a single size class within each species in order to reduce the potential for bias from differing seed sizes. Seeds used in this study were not treated with fungicides, insecticides or repellents prior to test initiation.

Test Soil

The soil used for the test represented a loam soil, and was composed of kaolinite clay, industrial quartz sand, and peat mixed in a 2:25:1 ratio (w:w:w). Crushed limestone was added to buffer the pH of the soil, and a slow-release fertilizer was added to provide nutrients essential for plant growth. A sample of soil representative of that used in this study was sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. The soil was determined to consist of 49% sand, 30% silt, and 21% clay, with an organic matter content of 2.1%. The soil pH was measured by Wildlife International Ltd. to be 7.79. A copy of the complete report from Agvise Laboratories, Inc. was filed in the archives at Wildlife International, Ltd. along with the raw data for this study.

Planting of Seeds

Seeds were planted in plastic pots (approximately 16 cm in diameter and 12 cm deep) on the day of test substance application. A template was used to gently compact the soil and leave ten uniform holes for planting. One indiscriminately selected seed was then planted in each hole, for a total of ten seeds in each pot. Holes were then closed by slightly depressing the soil surface.

Watering of Seedlings

Water lost through transpiration and evaporation was replaced by subirrigation with well water from the greenhouse facility. Seedlings were subirrigated to minimize the potential for the leaching of the test substance through the soil. Subirrigation trays were filled to a predetermined

depth to help standardize the amount of water delivered to each tray. The days on which watering occurred are listed in Appendix 5.

Environmental Conditions

The environmental conditions (temperature and relative humidity) of the test are summarized in Appendix 5. The temperature within the greenhouse was controlled with a Wadsworth MicroStep S/A Environmental Control System. Artificial lighting (high pressure sodium) was used to supplement natural sunlight in order to provide a uniform 14-hour photoperiod. The temperature and relative humidity within the greenhouse were continuously monitored during the test with the Wadsworth control system.

Pesticide and Metal Screening of Well Water and Soil

The well water and soil used for plant studies are analyzed periodically for pesticide and metals. No analytes were measured at levels that were expected to have an impact on the study. Reports for the latest analyses are stored in the archives at the Wildlife International, Ltd. site in Easton, Maryland.

Observations and Measurements

Observations on Days 7 and 14 were made primarily to document seedling emergence, although the general condition of test seedlings was assessed on Day 7. Observations on Day 21 were made to document seedling emergence and growth, and to determine the condition of individual seedlings following the application of the test substance. Observations consisted of noting whether emergence had or had not occurred, and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue at the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as necrosis, leaf wrinkle, chlorosis, plant lodging or plant stunting. Each emerged seedling was then assigned a numerical score (see Table 1) that described the plant condition (4). Condition score is a subjective or qualitative assessment that determines whether damage is slight, moderate, or severe. A score of 10 does not mean that 10% of the plant is showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.

The growth of emerged seedlings was evaluated by assessing the height and dry weight of living seedlings at test termination. Seedling height was measured to the nearest whole centimeter from the surface of the soil to the tip of the tallest leaf (corn, onion, and ryegrass) or to

the apical meristem (cucumber, soybean, and tomato). Seedlings were then clipped at soil level; the shoots of all living seedlings within a replicate were placed in a labeled bag, and dried. The total dry weight of the replicate was determined, and the mean dry weight per shoot was calculated by dividing the total weight by the number of seedlings weighed.

Analytical Sampling

On the day of test soil preparation (November 14, 2001), three soil samples were collected from the 20, 78, 313, 1250, and 5000 mg/kg treatment groups to verify the test concentrations and determine the homogeneity of the test substance in the carrier (soil). One sample was collected from the control group. Samples were stored at ambient room conditions until analysis was begun on November 15, 2001. Chemical analysis of the soil used in this study was performed by Wildlife International, Ltd. (Appendix 4). The test substance was used to prepare calibration standards.

Data Analyses

Statistical analyses were used to aid in the evaluation of effects of test substance application on seedling emergence, survival, mean shoot weight, and seedling height. These variables were defined for statistical analysis as follows:

Seedling Emergence:

The number of emerged seedlings per ten planted seeds in each pot.

Survival:

The number of emerged seedlings in each pot that were living at test termination per ten planted seeds.

Mean Shoot Weight:

The average dry shoot weight of living emerged seedlings in each pot.

Height:

The average height of living emerged seedlings in each pot.

Test data were evaluated to determine the no-observed-effect-concentration (NOEC) and lowest-observable-effect-concentration (LOEC) for condition and growth. The NOEC is defined as the maximum test substance concentration that shows no adverse phytotoxic effects and below which no phytotoxic effects are manifested. The LOEC is defined as the lowest test substance concentration used in the study that shows an adverse effect on a variable of interest. Mean seedling emergence, survival, weight, and height of the control and treatment groups were compared with Dunnett's t-test, using the DUNNETT option of the GLM (general linear model)

procedure of SAS version 8 (5). Significance was determined at the level of 0.05 ($p<0.05$). Dunnett's test was used to aid in establishing the NOEC by determining which treatment groups differed significantly from the control group.

Statistical analyses for species also included the determination of effect rates (EC estimates) and their confidence limits using the non-linear regression analysis of Bruce and Versteeg (6) when reductions in test endpoints among one or more treatment groups were 25% or more relative to control means. Analyses were conducted using the NLIN procedure of SAS version 8 (5). EC_x values (i.e. EC_{25} and EC_{50}) were defined as the test substance application rates that caused an x% change in the treatment group mean emergence, dry weight, or height relative to the control group. EC_x estimates were calculated using nominal test concentrations and treatment group mean values with the following equation:

$$R = \begin{cases} R_0 \cdot \Phi[(\log(EC_x)-\log(C))/\sigma+Z_x] & C>0 \\ R_0 & C=0 \end{cases}$$

where

R	= the predicted biotic response at concentration C
R_0	= the predicted biotic response for controls
$\Phi[\ ]$	= the cumulative area under the standard, normal distribution
$\log(EC_x)$	= the logarithm of the predicted ER giving an x percentage of decrease in the biological parameter vs. the control
Z_x	= the normal deviate above which x percentage of the area of the standard normal distribution lies.
σ	= the standard deviation of the normal distribution

Effects on survival were designated as LC_x values, and were calculated using the method described above.

RESULTS AND DISCUSSION

Analytical Chemistry

The results of analyses to measure concentrations of TBBPA in the soil samples collected during the test are presented in Appendix 4.

Biological Results

The results of the test are summarized for each species in Tables 2 through 7. Complete results are presented by species in Appendices 6 through 11.

Corn

There were no apparent adverse treatment-related effects on emergence, height or condition of corn seedlings. Mean emergence at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 9.25, 10.00, 9.25, 9.50, 9.25, and 9.75 seedlings per 10 planted seeds, respectively (Table 2). Mean height of seedlings at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 39.7, 44.1, 41.5, 39.2, 35.9, and 35.8 cm, respectively. None of the treatment group mean heights was significantly different ($p>0.05$) from the control means. The emerged seedlings generally appeared normal at test termination, excluding incidental mortality. The LC_{50} for corn seedlings, and the EC_{25} and the EC_{50} with respect to height was determined to be >5000 mg/kg soil dry weight.

There were apparent adverse treatment-related effects observed on dry weight of corn seedlings. The mean dry weight of seedlings in the control and the 20, 78, 313, 1250, 5000 mg/kg treatment groups was 0.45, 0.48, 0.45, 0.41, 0.36, and 0.36 g, respectively. The mean weights for the 1250 and 5000 mg/kg groups were significantly different ($p<0.05$) from the control mean. Therefore, the LOEC and NOEC for corn with respect to weight were 1250 mg/kg and 313 mg/kg, respectively. Since none of the treatment group weights was 25% less than the control mean, the EC_{25} and EC_{50} for corn weight were determined to be >5000 mg/kg.

Cucumber

There were no apparent adverse treatment-related effects on the emergence and condition of cucumber seedlings. Mean emergence at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 8.50, 9.50, 8.75, 8.75, 9.25, and 7.50 seedlings per 10 planted seeds, respectively (Table 3). The emerged seedlings generally appeared normal at test termination. There were isolated individuals with signs of leaf curl, necrosis, and mortality; however, none of these conditions appeared dose-responsive, and none were attributed to treatment. The LC_{50} for cucumber seedlings was determined to be >5000 mg/kg.

There were apparent adverse treatment-related effects on height and dry weight of cucumber seedlings. The mean height of seedlings at test termination in the control and the 20, 78, 313, 1250 and 5000 mg/kg treatment groups was 9.7, 9.4, 7.5, 6.5, 5.3, and 4.7 cm, respectively. Mean height of the 78, 313, 1250, 5000 mg/kg groups was significantly different ($p<0.05$) from the control mean. The LOEC and NOEC for cucumber seedlings with respect to height were 78 and 20 mg/kg, respectively. The EC_{25} for cucumber height was determined to be 131 mg/kg, and

the EC_{50} was determined to be 2603 mg/kg. The mean dry weight of seedlings in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 0.35, 0.32, 0.28, 0.20, 0.17, and 0.16 g, respectively. Mean weight of the 78, 313, 1250, and 5000 mg/kg groups was significantly different ($p<0.05$) from control means. The LOEC and NOEC for cucumber seedlings with respect to weight was 78 and 20 mg/kg, respectively. The EC_{25} for cucumber weight was determined to be 73 mg/kg, and the EC_{50} was determined to be 1672 mg/kg.

Onion

There were no apparent adverse treatment-related effects on seedling emergence, and condition of onion seedlings. The mean emergence of seedlings at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 9.00, 9.00, 9.50, 9.75, 9.25, and 9.00 seedlings per 10 planted seeds, respectively (Table 4). The emerged seedlings generally appeared normal at test termination, excluding incidental mortality. The LC_{50} was determined to be >5000 mg/kg.

There were apparent adverse treatment-related effects on the height and dry weight of onion seedlings. The mean height of seedlings at test termination for the control, and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 7.4, 7.9, 7.6, 7.2, 5.9, and 4.9 cm, respectively. Mean heights of the 1250 and 5000 mg/kg groups were significantly different ($p<0.05$) from the control means. The LOEC and the NOEC for onion seedlings with respect to height were 1250 and 313 mg/kg, respectively. The EC_{25} for onion height was determined to be 1948 mg/kg. Since none of the treatment group height were reduced by 50%, the EC_{50} was determined to be >5000 mg/kg. The mean weight of seedlings at test termination for the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 13, 13, 11, 10, 9, and 6 mg, respectively. Mean weights of the 1250 and 5000 mg/kg groups were significantly different ($p<0.05$) different from the control means. The LOEC and the NOEC for onion seedlings with respect to weight was 1250 and 313 mg/kg, respectively. The EC_{25} for onion weight was determined to be 460 mg/kg, the EC_{50} for onion weight was determined to be 4264 mg/kg.

Ryegrass

There were no apparent treatment-related effects on seedling emergence, and condition of ryegrass seedlings. The mean emergence of seedlings at test termination for the control, and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 9.25, 9.50, 9.25, 9.00, 8.75, and 8.25

seedlings per 10 planted seeds, respectively (Table 5). The emerged seedlings generally appeared normal, showing no signs of toxicity. The LC_{50} was determined to be >5000 mg/kg.

There were apparent adverse treatment-related effects on height and dry weight of ryegrass seedlings. Mean height at test termination for the control, and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 15.4, 15.7, 13.0, 9.6, 7.2, and 7.5 cm, respectively. Mean heights of the 313, 1250, and 5000 mg/kg groups were significantly different ($p<0.05$) from the control means. The LOEC and the NOEC for ryegrass seedlings with respect to height were 313 and 78 mg/kg, respectively. The EC_{25} for ryegrass dry weight was determined to be 114 mg/kg, the EC_{50} for ryegrass weight was determined to be 1801 mg/kg. Mean weights at test termination for the control, and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups were 21, 23, 17, 9, 7, and 7 mg, respectively. Mean weights of the 313, 1250, and 5000 mg/kg groups were significantly different ($p<0.05$) from the control means. The LOEC and NOEC for ryegrass seedlings with respect to weight were 313 and 78 mg/kg, respectively. The EC_{25} for ryegrass dry weight was determined to be 49 mg/kg, and the EC_{50} for ryegrass dry weight was determined to be 459 mg/kg.

Soybean

There were no apparent treatment-related effects on seedling emergence, height, dry weight, and condition of soybeans. Mean emergence at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 10.00, 9.50, 10.00, 10.00, 9.75, and 9.50 seedlings per 10 planted seeds, respectively (Table 6). Mean height at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 22.1, 22.7, 22.3, 22.4, 22.8, and 21.0 cm, respectively. None of the treatment group mean heights was significantly different ($p>0.05$) from the control means. The mean dry weight of seedlings in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 0.46, 0.48, 0.51, 0.47, 0.48, and 0.49 g, respectively. None of the treatment group mean weights was significantly different ($p>0.05$) from the control means. Since group mean weights and heights were not reduced by 25% (relative to the control mean), the EC_{25} and EC_{50} for weights and heights were determined to be >5000 mg/kg. The emerged seedlings generally appeared normal at test termination. There were a few individual plants that exhibited conditional ratings for leaf curl and stem curl; however, these cases were isolated and not considered treatment-related. The LC_{50} for soybean seedlings was therefore determined to be >5000 mg/kg.

Tomato

There were no apparent treatment-related effects on the emergence, and condition of tomato seedlings. The mean emergence at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 9.50, 8.75, 9.00, 9.25, 9.25, and 9.00 seedlings per 10 planted seeds, respectively (Table 7). Emerged seedlings generally appeared normal at test termination, excluding incidental mortality. The LC_{50} for tomato seedlings was determined to be >5000 mg/kg.

There were apparent treatment-related effects on height and dry weight of tomato seedlings. The mean height of seedlings at test termination for the control, and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 5.6, 5.0, 5.0, 4.8, 4.4, and 4.6 cm, respectively. Mean heights of the 1250 and 5000 mg/kg treatment groups were significantly different ($p<0.05$) from the control means. The LOEC and the NOEC for tomato seedlings with respect to height were 1250 and 313 mg/kg, respectively. The mean weight of seedlings at test termination in the control and the 20, 78, 313, 1250, and 5000 mg/kg treatment groups was 0.044, 0.039, 0.040, 0.033, 0.029, and 0.022 g, respectively. Mean weights of the 1250 and 5000 mg/kg treatment groups were significantly different ($p<0.05$) from the control means. The LOEC and the NOEC for tomato with respect to dry weight were 1250 and 313 mg/kg, respectively. The EC_{25} for tomato dry weight was determined to be 422 mg/kg, and the EC_{50} for tomato dry weight was determined to be >5000 mg/kg.

CONCLUSIONS

There were no adverse treatment-related effects on soybean seedling growth resulting from soil incorporated TBBPA. Therefore, the NOEC for soybean was the highest concentration tested, 5000 mg/kg, and the LOEC was not determined. Treatment-related effects on seedling growth were observed in corn, cucumber, onion, ryegrass, and tomato. The most sensitive endpoint for corn was seedling dry weight, which resulted in a NOEC of 313 mg/kg, and a LOEC of 1250 mg/kg. EC_{25} and EC_{50} for corn was determined to be >5000 mg/kg. The NOEC and LOEC for both seedling dry weight and height of cucumber – the most sensitive endpoints – were 20 and 78 mg/kg, respectively. The EC_{25} and EC_{50} values for dry weight of cucumber were determined to be 73 and 1672 mg/kg, respectively. The most sensitive endpoints for onion were seedling dry weight and height, which resulted in a NOEC of 313 mg/kg, and a LOEC of 1250 mg/kg. The EC_{25} and EC_{50} values for onion dry weight were determined to be 460 and 4264 mg/kg, respectively. The most sensitive endpoints for ryegrass were observed in dry weight and

height, which resulted in a NOEC of 78 mg/kg, and a LOEC of 313 mg/kg. The EC_{25} and EC_{50} values for dry weight were 49 mg/kg and 459 mg/kg, respectively. The most sensitive endpoints for tomato were observed in dry weight and height, which resulted in a NOEC of 313 mg/kg, and a LOEC of 1250 mg/kg. The value for the EC_{25} for tomato was determined to be 422 mg/kg; the EC_{50} was determined to be >5000 mg/kg.

REFERENCES

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2 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence).

3 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4225: Terrestrial Plant Toxicity, Tier II (Seedling Emergence).

4 **Frans, Robert E. and Ronald E. Talbert.** 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.

5 **SAS Institute, Inc.** 1999. SAS Proprietary Software Version 8 Cary, NC, SAS Institute, Inc.

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Table 1

Seedling Condition Rating System

The rating system below was used to help evaluate the health of seedlings on Day 21. Assigned scores by treatment group are reported on the following pages.

Rating	Category	Description
0	No Effect	No noticeable effect
10		Effect barely noticeable
20	Slight Effect	Some effect, not apparently detrimental
30		Effect more pronounced, not obviously detrimental
40		Effect moderate, plants appear able to recover
50	Moderate Effect	More lasting effect, recovery somewhat doubtful
60		Lasting effect, recovery doubtful
70		Heavy injury, loss of individual leaves
80	Severe Effect	Plant nearly destroyed, a few surviving leaves
90		Occasional surviving leaves
100	Complete Effect	Death of entire plant

Rating scale adapted from:

Frans, Robert E. and Ronald E. Talbert. 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 in B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.

Table 2

Effects of Tetrabromobisphenol A on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with CORN

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	9.00 ± 1.15	9.25 ± 0.96	9.25 ± 0.96	9.00 ± 1.15	0.45 ± 0.071	39.7 ± 4.57
20	10.00 ± 0.00 (-11%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-8%)	10.00 ± 0.00 (-11%)	0.48 ± 0.008 (-8%)	44.1 ± 1.04 (-11%)
78	9.00 ± 0.82 (0%)	9.25 ± 0.96 (0%)	9.25 ± 0.96 (0%)	9.00 ± 0.82 (0%)	0.45 ± 0.063 (-1%)	41.5 ± 3.38 (-5%)
313	9.00 ± 1.41 (0%)	9.50 ± 0.58 (-3%)	9.50 ± 0.58 (-3%)	9.25 ± 0.96 (-3%)	0.41 ± 0.036 (8%)	39.2 ± 2.60 (1%)
1250	9.00 ± 0.82 (0%)	9.25 ± 0.50 (3%)	9.25 ± 0.50 (0%)	9.25 ± 0.50 (-3%)	0.36 ± 0.038* (19%)	35.9 ± 1.86 (10%)
5000	9.75 ± 0.50 (-8%)	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-5%)	9.75 ± 0.50 (-8%)	0.36 ± 0.046* (20%)	35.8 ± 1.73 (10%)

* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Table 3

Effects of Tetrabromobisphenol A on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with CUCUMBER

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	8.50 ± 1.29	8.50 ± 1.29	8.50 ± 1.29	8.50 ± 1.29	0.35 ± 0.037	9.7 ± 1.11
20	8.25 ± 1.50 (3%)	9.50 ± 1.00 (-12%)	9.50 ± 1.00 (-12%)	8.75 ± 1.50 (-3%)	0.32 ± 0.014 (9%)	9.4 ± 0.53 (3%)
78	8.00 ± 1.15 (6%)	8.50 ± 1.29 (0%)	8.75 ± 1.26 (-3%)	8.25 ± 1.71 (3%)	0.28 ± 0.056* (20%)	7.5 ± 1.72* (22%)
313	8.25 ± 1.71 (3%)	8.75 ± 1.26 (-3%)	8.75 ± 1.26 (-3%)	8.50 ± 1.29 (0%)	0.20 ± 0.035* (42%)	6.5 ± 1.21* (33%)
1250	9.00 ± 1.41 (-6%)	9.00 ± 1.41 (-6%)	9.25 ± 1.50 (-9%)	9.25 ± 1.50 (-9%)	0.17 ± 0.011* (50%)	5.3 ± 0.46* (45%)
5000	6.75 ± 2.06 (21%)	7.50 ± 2.38 (12%)	7.50 ± 2.38 (12%)	7.50 ± 2.38 (12%)	0.16 ± 0.020* (54%)	4.7 ± 0.53* (52%)

* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Table 4

Effects of Tetrabromobisphenol A on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with ONION

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (mg) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	8.50 ± 1.29	9.00 ± 1.41	9.00 ± 1.41	9.00 ± 1.41	0.013 ± 0.0021	7.4 ± 0.78
20	9.00 ± 1.15 (-6%)	9.00 ± 1.55 (0%)	9.00 ± 1.55 (0%)	8.25 ± 1.71 (8%)	0.013 ± 0.0034 (1%)	7.9 ± 0.85 (-7%)
78	9.25 ± 0.50 (-9%)	9.50 ± 0.58 (-6%)	9.50 ± 0.58 (-6%)	9.50 ± 0.58 (-6%)	0.011 ± 0.0020 (15%)	7.6 ± 0.46 (-3%)
313	9.50 ± 0.58 (-12%)	9.75 ± 0.50 (-8%)	9.75 ± 0.50 (-8%)	9.75 ± 0.50 (-8%)	0.010 ± 0.0027 (21%)	7.2 ± 0.62 (2%)
1250	9.00 ± 0.82 (-6%)	9.25 ± 0.50 (-3%)	9.25 ± 0.50 (-3%)	9.00 ± 0.00 (0%)	0.009 ± 0.0019* (33%)	5.9 ± 0.31* (21%)
5000	8.25 ± 0.96 (3%)	9.00 ± 0.00 (0%)	9.00 ± 0.00 (0%)	8.50 ± 0.58 (6%)	0.006 ± 0.0012* (52%)	4.9 ± 0.28* (33%)

* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Table 5

Effects of Tetrabromobisphenol A on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with RYEGRASS

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (mg) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	9.00 ± 0.82	9.25 ± 0.96	9.25 ± 0.96	9.25 ± 0.96	0.021 ± 0.0012	15.4 ± 1.29
20	8.75 ± 1.26 (3%)	9.50 ± 0.58 (-3%)	9.50 ± 0.58 (-3%)	9.50 ± 0.58 (-3%)	0.023 ± 0.0056 (-8%)	15.7 ± 1.85 (-2%)
78	9.25 ± 0.96 (-3%)	9.00 ± 0.82 (3%)	9.25 ± 0.50 (0%)	9.25 ± 0.50 (0%)	0.017 ± 0.0066 (21%)	13.0 ± 1.56 (16%)
313	8.75 ± 0.96 (3%)	9.00 ± 0.82 (3%)	9.00 ± 0.82 (3%)	9.00 ± 0.82 (3%)	0.009 ± 0.0039* (55%)	9.6 ± 1.75* (37%)
1250	8.00 ± 1.63 (11%)	8.50 ± 1.73 (8%)	8.75 ± 1.89 (5%)	8.75 ± 1.89 (5%)	0.007 ± 0.0030* (65%)	7.2 ± 1.15* (53%)
5000	7.75 ± 2.06 (14%)	8.25 ± 1.26 (11%)	8.25 ± 1.26 (11%)	8.25 ± 1.26 (11%)	0.007 ± 0.0032* (67%)	7.5 ± 0.93* (51%)

* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Table 6

Effects of Tetrabromobisphenol A on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with SOYBEAN

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	9.50 ± 0.58	10.00 ± 0.00	10.00 ± 0.00	10.00 ± 0.00	0.46 ± 0.014	22.1 ± 2.03
20	9.00 ± 0.82 (5%)	9.50 ± 0.58 (5%)	9.50 ± 0.58 (5%)	9.50 ± 0.58 (5%)	0.48 ± 0.041 (-3%)	22.7 ± 1.60 (-3%)
78	9.25 ± 0.50 (3%)	10.00 ± 0.00 (0%)	10.00 ± 0.00 (0%)	10.00 ± 0.00 (0%)	0.51 ± 0.028 (-10%)	22.3 ± 2.09 (-1%)
313	9.50 ± 0.58 (0%)	10.00 ± 0.00 (0%)	10.00 ± 0.00 (0%)	10.00 ± 0.00 (0%)	0.47 ± 0.051 (-1%)	22.4 ± 2.81 (-1%)
1250	9.50 ± 0.58 (0%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	9.75 ± 0.50 (3%)	0.48 ± 0.054 (-3%)	22.8 ± 2.11 (-3%)
5000	9.00 ± 0.00 (5%)	9.25 ± 0.50* (8%)	9.50 ± 0.58 (5%)	9.50 ± 0.58 (5%)	0.49 ± 0.049 (-7%)	21.0 ± 1.86 (5%)

* Treatment group mean was significantly different from the control mean (Dunnett's test, $p<0.05$)

Table 7

Effects of Tetrabromobisphenol A on Seedling Emergence, Survival, Shoot Dry Weight, and Height in a 21-Day Seedling Emergence Test with TOMATO

Test Concentration (mg/kg)	Number of Emerged Seedlings (% Reduction) Day 7	Day 14	Day 21	Seedling Survival (% Reduction)	Dry Weight (g) (% Reduction)	Seedling Height (cm) (% Reduction)
Control	5.00 ± 2.31	9.50 ± 0.58	9.50 ± 0.58	9.25 ± 0.50	0.044 ± 0.0088	5.6 ± 0.38
20	7.75 ± 2.06 (-55%)	8.75 ± 0.96 (8%)	8.75 ± 0.96 (8%)	8.75 ± 0.96 (5%)	0.039 ± 0.0115 (12%)	5.0 ± 0.84 (11%)
78	4.75 ± 1.26 (5%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (3%)	0.040 ± 0.0087 (9%)	5.0 ± 0.74 (11%)
313	7.50 ± 1.73 (-50%)	9.00 ± 0.00 (5%)	9.25 ± 0.50 (3%)	9.00 ± 0.82 (3%)	0.033 ± 0.0065 (26%)	4.8 ± 0.20 (13%)
1250	4.75 ± 2.87 (5%)	9.25 ± 1.50 (3%)	9.25 ± 1.50 (3%)	9.25 ± 1.50 (0%)	0.029 ± 0.0055* (34%)	4.4 ± 0.56* (21%)
5000	6.25 ± 1.71 (-25%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (5%)	9.00 ± 0.82 (3%)	0.022 ± 0.0031* (50%)	4.6 ± 0.21* (18%)

* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Table 8

Observed NOEC and Calculated ECx Estimates for
Tetrabromobisphenol A on Seedling Emergence, Survival, Height, and Dry Weight
in a 21-Day Seedling Emergence Test

Species	Endpoint (NOEC) (mg/kg)		Estimate	Lower 95% CL (mg/kg)	Upper 95% CL
Corn	Emergence	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Survival	LC_{25}	>5000	-	-
	(5000)	LC_{50}	>5000	-	-
	Height	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Dry Weight	EC_{25}	>5000	-	-
	(313)	EC_{50}	>5000	-	-
Cucumber	Emergence	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Survival	LC_{25}	>5000	-	-
	(5000)	LC_{50}	>5000	-	-
	Height	EC_{25}	131	8.03	2150
	(20)	EC_{50}	2600	506	13400
	Dry Weight	EC_{25}	72.6	1.74	3040
	(20)	EC_{50}	1670	194	14400
Onion	Emergence	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Survival	LC_{25}	>5000	-	-
	(5000)	LC_{50}	>5000	-	-
	Height	EC_{25}	1950	668	5680
	(313)	EC_{50}	>5000	-	-
	Dry Weight	EC_{25}	460	150	1410
	(313)	EC_{50}	4260	2240	8140
Ryegrass	Emergence	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Survival	LC_{25}	>5000	-	-
	(5000)	LC_{50}	>5000	-	-
	Height	EC_{25}	114	0.871	14900
	(78)	EC_{50}	1800	111	29300
	Dry Weight	EC_{25}	48.5	0.202	11700
	(78)	EC_{50}	459	16.3	12900
Soybean	Emergence	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Survival	LC_{25}	>5000	-	-
	(5000)	LC_{50}	>5000	-	-
	Height	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Dry Weight	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
Tomato	Emergence	EC_{25}	>5000	-	-
	(5000)	EC_{50}	>5000	-	-
	Survival	LC_{25}	>5000	-	-
	(5000)	LC_{50}	>5000	-	-
	Height	EC_{25}	>5000	-	-
	(313)	EC_{50}	>5000	-	-
	Dry Weight	EC_{25}	422	81.2	2200
	(313)	EC_{50}	5460	1960	15200

Appendix 1

Personnel Involved In the Study

The following key personnel were involved in the conduct or management of this study:

(1) Henry O. Krueger, Ph.D., Director, Aquatic Toxicology and Non-Target Plants
(2) John R. Porch, Supervisor, Non-Target Plants and Insects
(3) Andrew J. Brignole, Biologist
(4) Timothy Z. Kendall, Supervisor
(5) Willard B. Nixon, Director, Analytical Chemistry

Appendix 2

Study Protocol and Deviations

PROTOCOL

TETRABROMOBISPHENOL A: A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.4100 and 850.4225

and

OECD Guideline for Testing of Chemicals
Proposal for Revision of Guideline 208: Terrestrial Non-Target Plant Tests

Submitted to

American Chemistry Council's
Brominated Flame Retardant Industry Panel
1300 Wilson Boulevard
Arlington, Virginia 22209

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

September 5, 2001

Wildlife International, Ltd.

TETRABROMOBISPHENOL A: A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF SIX SPECIES OF PLANTS

SPONSOR:	American Chemistry Council's Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209
SPONSOR'S REPRESENTATIVE:	Ms. Wendy Sherman
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	John R. Porch Senior Biologist
LABORATORY MANAGEMENT:	Henry O. Krueger, Ph.D. Director of Aquatic Toxicology & Non-Target Plants

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: November 27, 2001 Experimental Termination Date: December 18, 2001

Project No.: 439-102

Test Concentrations: 0 (control), 20, 78, 313, 1250, 5000 mg/kg

Test Substance No.: 5754 Reference Substance No. (if applicable): N/A

PROTOCOL APPROVAL

STUDY DIRECTOR	DATE: 7 Nov 2001
LABORATORY MANAGEMENT	DATE: 11/6/01
SPONSOR'S REPRESENTATIVE	DATE: September 10, 2001

PROTOCOL NO. 439/090401/SEEDEM-10/SU439

Wildlife International, Ltd.

INTRODUCTION

Wildlife International, Ltd. will conduct a toxicity test with six species of plants to determine the effects of a test substance on seedling emergence and early growth. The test will be conducted at the Wildlife International, Ltd. plant testing facility near Easton, Maryland. The six species to be tested include rye grass, onion, corn, soybean, cucumber, and tomato. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4100 (1) and 850.4225 (2) and in the OECD Guideline for Testing of Chemicals: Proposal for Revision of Guideline 208: "Terrestrial Non-target Plant Tests" (3). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the effect of Tetrabromobisphenol A (TBBPA) on the seedling emergence and growth of six species of plants.

EXPERIMENTAL DESIGN

The target test concentration(s) will be selected by the Sponsor in consultation with Wildlife International, Ltd., and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. If necessary, the test concentrations to be used for each species will be added to the protocol by amendment.

For each plant species tested, seeds will be planted and exposed to a series of five concentrations of the test substance. A negative control and, if appropriate, a solvent control group will be maintained concurrently. There will be four replicates for each treatment and control group. Each replicate will consist of a growth pot containing ten seeds. The replicates will be placed on a benchtop in a greenhouse according to a randomized design. Data collected from all replicates within a treatment group will be combined for calculating EC25 and EC50 values, as well as the no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC).

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One application of each of the various treatments will be made by soil incorporation of the test substance prior to planting seeds. The duration of the in-life portion of the test will be 21 days following planting, during which time possible phytotoxic effects of the test substance on seedling emergence and growth of emerged seedlings will be evaluated.

MATERIALS AND METHODS

Test Substance

The test substance consisted of a composite of TBBPA samples received from three manufacturers. The material's identity and date received from each of the manufacturers is given below:

Manufacturer	Lot/Batch	Date Received	Wildlife International Ltd. Identification Number
Great Lakes Chemical Corporation	1008JE04B	August 16, 2001	5722
Albemarle Corporation	25115T-1	August 16, 2001	5721
Bromine Compounds, Ltd.	010040	August 31, 2001	5733

The composite test substance was assigned Wildlife International Ltd. identification number 5754 and was stored under ambient conditions.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study.

Test Soil Preparation

Concentrations of the test substance in the soil will be prepared on a dry weight basis (e.g., mg test chemical/kg dry soil). The test substance will be incorporated into the soil for each treatment level prior to planting.

Species to be Tested

The six species of plants used in this study were chosen because they are economically important, and are readily cultivated test organisms that are widely used in research. The common and scientific names for the species and their approximate planting depths are listed below:

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Monocots:		Planting Depth
Rye Grass	*Lolium perenne*	6 mm
Onion	*Allium cepa*	6 mm
Corn	*Zea mays*	2.0 - 2.5 cm
Dicots:		
Soybean	*Glycine max*	2.0 - 2.5 cm
Cucumber	*Cucumis sativa*	2.0 - 2.5 cm
Tomato	*Lycopersicon esculentum.*	6 mm

Seeds will be selected from a single size class within each species. The seeds of most plant species are sorted according to size by the supplier prior to being obtained by Wildlife International, Ltd. However, in some cases it may be necessary to further sort seeds to form a more uniform size class that reduces the potential for bias from differing seed sizes.

Seeds used in this study will not have been treated with fungicides, insecticides or repellents prior to test initiation. Seeds will be obtained from a producer or supplier such as Meyer Seed Company, Baltimore, Maryland. Any documentation provided from the supplier concerning the identification and history of the seeds used will be included in the study data.

Test Soil

Test plants will be grown in pots with a sandy-loam soil substrate. Analyses will be performed at least once annually to characterize the soil. A sample of soil representative of that used in this study will be sent to Agvise Laboratories, Inc., in Northwood, North Dakota, for analysis of the particle size distribution and organic matter content of the soil. Soil characterization will include, but may not be limited to, the determination of particle size distribution, organic matter content, and pH. Those items relevant to the conduct of the study will be discussed in the final report. The complete report from Agvise Laboratories, Inc. will be filed in the archives located at Wildlife International, Ltd. The results of the characterization will be stored in the archives located at the Wildlife International, Ltd. site, and those items relevant to the conduct of the study will be discussed in the final report.

PROTOCOL NO. 439/090401/SEEDEM-10/SU439

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Pesticide and Metal Screening

Neither the well water nor the artificial soil are expected to have contaminants present in quantities known to be capable of interfering with the study. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of water and soil used in this study. Results of the analyses will be stored in the archives located on the Wildlife International Ltd. site.

Environmental Conditions

The test will be conducted within a greenhouse. Environmental conditions, including temperature and light intensity, will be controlled using a Wadsworth MicroStep/SA environmental control system. Temperature and relative humidity in the study room will be continuously monitored with a Campbell Scientific data logger, and daily conditions throughout the test will be reported. A photoperiod of at least 14 hours light will be maintained during the test. Artificial lighting may be used to lengthen short-day photoperiods or to supplement natural sunlight on overcast days.

Test Procedure

Growth pots will be filled with test or control soil, and ten seeds of one species will be planted per replicate. The seeds will be planted at the appropriate depth and will be approximately equally spaced. Seeds will be assigned to test and control groups and planted in growth pots uniquely identified with a minimum of the species name, project number, treatment group designation, and replicate. This method of application was chosen because contaminated soil is the most likely route of exposure to plants. After planting, the growth pots will be placed on benches in the greenhouse in a randomized configuration to minimize bias from microclimates which may exist within the greenhouse. Initial watering will be done to the soil surface after planting. Thereafter, water will be supplied to the growth pots by sub-irrigation to help ensure that sufficient water is available for seedling growth. Records of the days that watering occurs and source of water used will be kept in the study data.

The growth pots will be observed weekly after test initiation in order to determine the number of emerged seedlings. The in-life portion of the test will terminate twenty-one days after initiation, however, the test may be extended at the discretion of the study director for one or more species. If any portion of the test is extended, the duration of and the reason for the extension will be documented in the data and discussed in the final report. At the termination of the in-life portion of

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the test, height measurements and the condition of the emerged seedlings will be recorded. The height of each living seedling within a replicate will be determined in order to calculate the mean seedling height per replicate. The exact method used to measure height may vary with species, and will be described in the raw data and included in the final report.

At the in-life phase termination, the condition of seedlings will be assessed utilizing a rating system based upon Frans and Talbert (4). A numerical rating will be assigned to help characterize changes in the seedlings' morphology including necrosis, chlorosis, general development, or any other characteristic that may be deemed a response of the seedling to the treatment. Ratings may range from 0 to 100, 0 indicating normal seedling appearance, 100 indicating emerged seedlings that have died prior to test termination. Intermediate scores reflect the severity of changes in plant condition. After final observations are completed, plants will be clipped at soil level and the above-ground portion (shoots) of all living plants within each replicate will be dried to a constant weight. The mean shoot dry weight of each replicate will be calculated.

Sampling for Analytical Measurements

On each day of test substance application, samples of the test soils will be collected for the analysis of the test substance. Samples will be placed in an appropriate storage container (e.g., glass or polypropylene bottles) and stored under conditions designated by the Sponsor until analyzed. Triplicate samples will be collected from the soil of each test concentration to verify concentrations and demonstrate homogeneity in the soil.

Experimental Group	Day 0
Control	1
Solvent Control (if needed)	1
Level 1-Low Concentration	3
Level 2	3
Level 3	3
Level 4	3
Level 5-High Concentration	3
Total Number of Samples = 17	

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The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical validation phase of the study.

Analytical Method Development and Verification

Wildlife International, Ltd. will develop appropriate analytical methods and validate them for Sponsor approval prior to their use in support of this study. If the Sponsor provides an analytical method, Wildlife International, Ltd. will demonstrate its validity to the Sponsor before being used in support of this study. All analytical methods accepted for use in this study will be added by protocol amendment and described in detail as an Appendix to the final report."

Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International, Ltd. using High Performance Liquid Chromatography (HPLC). The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report. Maximum sample holding times, prior to analysis, will not exceed one week from the date of the collection of samples.

Data Analyses

This section includes proposed statistical analyses. Additional tests or analyses may be performed when warranted at the discretion of the Study Director or by Sponsor request.

An evaluation of potential effects of the test substance on seedling emergence, the growth of emerged seedlings, as characterized by shoot weight and height, and seedling condition will be made. Statistical analyses will include the determination of effect concentrations (EC estimates), and the determination of which treatment groups differ significantly from the control group(s).

The 25 and 50% effect concentrations and their 95% confidence intervals will be determined when warranted using an appropriate technique, such as Probit analysis or linear interpolation. When possible, EC estimates will be made for mean seedling emergence, mean shoot weight and height of seedlings at test termination.

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The data will be evaluated to determine the lowest-observed-effect concentration (LOEC), defined as the lowest concentration of test substance used in the study that shows an adverse effect on a variable of interest. The no-observed-effect-concentration (NOEC) will be defined as the maximum concentration which shows no adverse phytotoxic effects and below which no phytotoxic effects are manifested. Dunnett's two-tailed test will be used to determine significant differences from the control(s) at the 0.05 level of significance. Significant differences from the control, or their absence, may help establish the LOEC and NOEC.

All statistical analyses will be performed on a personal computer using commercially available statistical software programs (5, 6). The specific statistical tests and the programs used to perform the tests will be described in the final report of the study.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International, Ltd. will include but not be limited to:

1. Copy of signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Test soil calculation and preparation.
5. Observations.
6. The methods used to analyze test substance concentrations and the results of analytical measurements.
7. Statistical calculations, if applicable.
8. Test conditions (temperature, humidity, etc.).
9. Calibration records for application equipment.
10. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but not be limited to, the following, when applicable.

1. Name and address of the facility performing the study.

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2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. The test substance identification including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
5. A copy of the protocol and protocol amendments.
6. Stability and solubility of the test substance under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test species, including the source and scientific name.
9. A description of the preparation of the test solutions.
10. The methods used to allocate seeds to test substrates and begin the test, the number of seeds and replicates per treatment, and the duration of the test.
11. A description of circumstances that may have affected the quality or integrity of the data.
12. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
13. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
14. Statistical methods used to evaluate the data.
15. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
16. The location where raw data and final report are to be stored.
17. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
18. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.

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CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with Good Laboratory Practice Standards for EPA (40 CFR Part 160 and/or Part 792); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1 October 1999). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site or at an alternative location to be specified in the final report.

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REFERENCES

1 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence).

2 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4225: Terrestrial Plant Toxicity, Tier II (Seedling Emergence).

3 **OECD.** 1998. *Guideline for Testing of Chemicals, Proposal for Revision of Guideline 208: Terrestrial Non-target Plant Tests.* Organization for Economic Cooperation Development.

3 **Fraas, Robert E. and Ronald E. Talbert.** 1977. Design of Field Experiments and the Measurement and Analysis of Plant Responses. Pages 15-23 *in* B. Truelove, ed. Research Methods in Weed Science. Southern Weed Science Society, Auburn University, Alabama.

4 **SAS Institute, Inc.** 1989. SAS/STAT User's Guide , Version 6, Fourth Edition, Volume 1, Cary, NC, SAS Institute, Inc., 943 pp.

5 **Norberg-King, T.J.** 1993. *A Linear Interpolation Method for Sublethal Toxicity: The Inhibition Concentration (ICp) Approach (Version 2.0).* U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota.

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APPENDIX I

IDENTIFICATION OF TEST SUBSTANCE BY SPONSOR

To be Completed by Sponsor

I. Test Substance Identity (name to be used in the report): Tetrabromobisphenol-A

Reference Standard (if applicable): N/A

Test Substance Sample Code or Batch Number: Wildlife International, Ltd. No.5381

Test Substance Purity (% Active Ingredient): 98.91% Expiration Date: August 1, 2002

II. Test Substance Characterization

Have the identity, strength, purity and composition or other characteristics which appropriately define the test substance and reference standard been determined prior to its use in this study in accordance with GLP Standards? X Yes ___ No

III. Test Substance Storage Conditions

Please indicate the recommended storage conditions at Wildlife International, Ltd..

Ambient temperature; protect from light and moisture

Has the stability of the test substance under these storage conditions been determined in accordance with GLP Standards? X Yes ___ No

Other pertinent stability information:

N/A

IV. Toxicity Information:

Mammalian: Rat LD50 > 5 g/kg Mouse LD50: > 10 g/kg

Aquatic:	Invertebrate Toxicity (EC/LC50)	Fish Toxicity (LC50)
	N/A	N/A

Other Toxicity Information (including findings of chronic and subchronic tests):

V. Classification of the Compound:

_____Insecticide _____Herbicide _____Fungicide

_____Microbial Agent _____Economic Poison

Other: Halogenated flame retardant

DEVIATION FROM STUDY PROTOCOL

STUDY TITLE: Tetrabromobisphenol A: A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants

PROTOCOL NO.: 439/090401/SEEDEM-10/SU439 **DEVIATION NO.:** 1

SPONSOR: American Chemistry Council's Brominated Flame Retardant Industry Panel **PROJECT NO.:** 439-102

DATE(S) OF DEVIATION: As noted below

DEVIATION: November 14, 2001
Eleven seeds were planted in one replicate (Tomato 1250 mg/kg, C).

REASON:
Oversight. The replicate means were appropriately adjusted for the presence of the eleventh seedling. There will be no adverse impact on the study as a result of this deviation.

DEVIATION: November 14, 2001
The artificial soil used for the test was classified as a loam rather than a sandy-loam.

REASON:
Results of the soil characterization indicated that the sand content was slightly less than anticipated. There will be no adverse impact on the study as a result of this deviation.

DEVIATION: November 14, 2001
The initial watering for the test was not made on the soil surface.

REASON:
Surface watering is used to help surface-applied test substances move into the soil column. The procedure is not necessary for soil-incorporated applications. There will be no adverse impact on the study as a result of this deviation.

DEVIATION: November 21, 2001
Observations made on Day 7 included a general assessment of seedling condition.

REASON:
Oversight. Seedling conditions are routinely observed to monitor the progress of the test. There will be no adverse impact on the study as a result of this deviation.

DEVIATION: November 14, 2001

The analytical method was not amended to the protocol.

REASON:

The protocol states in the "Analytical Method Development and Verification" section that the analytical methods used would be amended to the protocol. However, the next section, "Analytical Methods," clearly identifies the method as HPLC. The first reference should have been removed from the protocol upon the inclusion of the "Analytical Methods" section. There will be no adverse impact on the study as a result of this deviation.

STUDY DIRECTOR — DATE: 5 March 02

LABORATORY MANAGEMENT — DATE: 5 March 02

Appendix 3
Certificate of Analysis

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ALBEMARLE CORPORATION
RESEARCH AND DEVELOPMENT DEPARTMENT

FINAL REPORT ON THE CHEMICAL CHARACTERIZATION (IDENTITY, PURITY AND HOMOGENEITY) OF TETRABROMOBISPHENOL-A (TBBPA), WIL TEST SUBSTANCE 5754, COMPOSITED FROM WIL 5733, 5721 AND 5722

I.	Reference Protocol Number:	TBBPA-10-01-2001
II.	Sponsor:	American Chemistry Council Brominated Flame Retardant Industry Panel 1300 Wilson Boulevard Arlington, Virginia 22209 Study Monitor: Wendy K. Sherman
III.	Analytical Testing Facilities:	Albemarle Corporation Albemarle Technical Center 8000 GSRI Avenue Baton Rouge, LA 70820 Study Chemist: Paul F. Ranken, Ph. D.
IV.	Dates of Performance:	Study Initiation Date: September 28, 2001 Study Completion Date: November 2, 2001
V.	Test Article:	Tetrabromobisphenol-A (WIL Test Substance 5754). The test article is a composite of WIL #5733, 5721, and 5722 which are samples of commercial products from Albemarle Corporation, Great Lakes Chemical Corporation and the Dead Sea Bromine Group. The composite was prepared by Wildlife International Ltd., Easton, MD 21601.
VI.	Objective/Methodology:	This study was initiated to confirm the identity of the test article, to demonstrate the homogeneity of the test article and to demonstrate the purity of the test article. The identity of one sample of the test article, designated Characterization Sample, was confirmed by Fourier Transform Infrared Spectroscopy using SOP No. ARS-284-R4. In this procedure, the sample infrared spectrum was compared to a standard reference spectrum

of TBBPA. The homogeneity of the test article was demonstrated by determining the purity of six separate test article samples which were taken from the top, middle and bottom of the right side of the bulk container and from the top, middle and bottom of the left side of the bulk container. The purity (area % TBBPA) of the six samples was determined by High Performance Liquid Chromatography (HPLC) using SOP No. ARS-443-R1. The homogeneity of the test article was established by demonstrating that all six samples had the same purity (< 5% difference of the TBBPA area % for each sample compared to the average TBBPA area % of the six samples). The six test article samples were further characterized by measuring the concentration (area%) of three potential impurities: tribromophenol, tribromobisphenol-A and o,p'-tetrabromobisphenol-A. Chain of Custody and sample handling were conducted according to established standard operating procedures.

VII. Results:

Table 1 and table 2 contain the test article analytical data from the study. The identity of the test article was confirmed by Fourier Transform Infrared Spectroscopy. The homogeneity of the test article was confirmed by HPLC analysis; all six test article samples had the same purity (<5% difference of the TBBPA area% for each sample compared to the average TBBPA area% of the six samples). Further characterization of the six test article samples was accomplished by measuring the concentration of three expected impurities. There were no circumstances that may have affected the quality or integrity of the data.

VIII. Regulatory Requirements:

The study conformed to the requirements of EPA TSCA (40 CFR Part 792) Good Laboratory Practice Regulations and the

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OECD [C(97)186/Final] Good Laboratory Practice Regulations.

IX. Data/Record Retention:

All original log books, spectra and reports will be forwarded to the Quality Assurance Unit (QAU) for a final review prior to filing in the designated Health and Environment archives at Albemarle Corporation, Health and Environment Department, 451 Florida Street, Baton Rouge, LA 70801.

Paul F. Ranken November 2, 2001

Paul F. Ranken, Ph. D. DATE
STUDY CHEMIST

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CONCLUSIONS AND TEST ARTICLE ANALYTICAL DATA. 1.

CHEMICAL NAME: Tetrabromobisphenol-A
C.A.S. No.: 79-94-7
MOLECULAR FORMULA: $C_{15}H_{10}Br_4O_2$
PHYSICAL FORM: White Powder
CHEMICAL STRUCTURE:

ANALYSIS	RESULTS		ANALYSIS DATES	ANALYST
FT-IR	The sample FT-IR spectrum matched that of the reference spectrum. All spectra are on file with the original data.		10/12/01	W. T. Cobb
HPLC				
Sample	Purity (area% TBBPA)	Average	Difference (%) from average	
middle right	99.14	99.17	<5%	10/25/01 J. S. Arroyave
middle left	99.18	99.17	<5%	10/25/01 J. S. Arroyave
bottom right	99.09	99.17	<5%	10/25/01 J. S. Arroyave
top right	99.19	99.17	<5%	10/25/01 J. S. Arroyave
top left	99.16	99.17	<5%	10/25/01 J. S. Arroyave
bottom left	99.23	99.17	<5%	10/25/01 J. S. Arroyave
CONCLUSION: Based on these analytical data, the test article identity was confirmed as tetrabromobisphenol-A. The composite sample was shown to be homogeneous with a purity of 99.17%.				

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Conclusions and Test Article Data. 2.

Characterization of Test Article by HPLC (Area%)

	TBBPA	Tribromophenol	TriBPA	o,p'-TBBPA
Middle Right	99.14	Not detected	0.82	0.04
Middle Left	99.18	Not detected	0.76	0.06
Bottom Right	99.09	Not detected	0.84	0.06
Top Right	99.19	Not detected	0.78	0.04
Top Left	99.16	Not detected	0.8	0.04
Bottom Left	99.23	Not detected	0.72	0.04

Appendix 4

Analysis of Tetrabromobisphenol A (TBBPA) in Soil Samples From a Seedling Emergence Test

Analytical Method

The method used for the analysis of Tetrabromobisphenol A (TBBPA) in soil samples was developed by Wildlife International, Ltd. Samples were processed as follows:

Soil samples (10.0 g) were weighed into French square bottles. To each sample, 100 mL of methanol acidified with phosphoric acid was added and samples were sonically disrupted for approximately 10 minutes at a setting of approximately 60. Following disruption the samples were allowed to cool undisturbed for approximately 5 minutes. An aliquot of each sample was transferred into a microcentrifuge tube and centrifuged for approximately 5 minutes. The methanolic extract was volumetrically diluted using 50% methanol : 50% NANOpure® water. An aliquot of each diluted extract was transferred to an autosampler vial for immediate HPLC analysis. A method flowchart for the analysis of sediment samples is provided in Figure 1

Concentrations of TBBPA were determined by high performance liquid chromatography using a Hewlett-Packard Model 1100 High Performance Liquid Chromatograph (HPLC) equipped with an Agilent Series 1100 Variable Wavelength Detector. Chromatographic separations were achieved using a YMC Pack ODS AM analytical column (150 x 4.6 mm, 3 µm particle size). The instrument parameters are summarized in Table 1 and a method flow chart is provided in Figure 1.

Calibration Curve

Calibration standards containing TBBPA ranging from 0.100 to 1.00 mg/L were prepared in 50% methanol : 50% NANOpure® water and analyzed with the sample set. A linear regression analysis was generated using the peak area responses versus the respective concentrations of the calibration standards. A representative calibration curve for TBBPA is presented in Figure 2. The concentration of TBBPA in the samples was determined by substituting the peak area responses into the linear regression equation. Representative chromatograms of low and high calibration standards for TBBPA are presented in Figures 3 and 4, respectively.

Example Calculations

A sample calculation of sample number 439-102-16 having a nominal concentration of 5000 mg/Kg TBBPA in the soil follows:

Initial mass (M_1) : 10.0 grams
Initial final volume (V_1): 100 mL
Secondary dilution (V_2): 0.150 → 100 mL
Dilution Factor (V_1/M_1) x (V_2) = 6667
Peak Area: 43.89433
Linear regression equation:
Slope: 60.9319
$Y_{Intercept}$: -0.2275

$$\text{Concentration TBBPA (mg/Kg)} = \frac{(\text{Peak area} - Y_{intercept}) \times \text{Dilution factor}}{\text{Slope}}$$

$$\text{Concentration TBBPA (mg/Kg)} = \frac{(43.89433 + 0.2275) \times 6667}{60.9319}$$

$$\text{Concentration TBBPA (mg/Kg)} = 4828 \text{ mg/Kg}^*$$

$$\text{Percent of Nominal Conc.} = \frac{4828 \text{ mg/Kg}}{5000 \text{ mg/Kg}} \times 100$$

$$\text{Percent of Nominal Conc.} = 96.6\%^*$$

* Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

RESULTS

Sample Analysis

Soil samples were collected from a study designed to determine the effects of Tetrabromobisphenol A (TBBPA) on the seedling emergence of non-target plants. Samples were processed on November 15, 2001 and analyzed on November 15 and 16, 2001. Concentrations of TBBPA in soil in the range of 20 to 5000 mg/Kg yielded percent recoveries from 67.3 to 96.6%. The mean percent recoveries of triplicate samples at 20, 78, 313, 1250 and 5000 mg/Kg were 80.0, 82.1, 80.8, 84.7 and 91.9%, respectively. Quality control samples fortified at 15.0, 100 and 5000 mg/Kg yielded percent recoveries of 97.9, 94.9 and 99.4%, respectively. The control sample was devoid of TBBPA. Analytical results for all exposure and quality control samples are presented in Table 2. A chromatogram of a control sample (439-102-1) is presented in Figure 5. A representative chromatogram of a soil extract (439-102-16) is presented in Figure 6.

Table 1

HPLC Operational Parameters

INSTRUMENT:	Hewlett-Packard Model 1100 High Performance Liquid Chromatograph with an Agilent Series 1100 Variable Wavelength Detector			
ANALYTICAL COLUMN:	YMC Pack ODS AM (150 mm x 4.6 mm, 3 µm particle size)			
FLOW RATE:	1.000 mL/min			
OVEN TEMPERATURE:	40°C			
MOBILE PHASE:	Solvent A: 0.1% H_3PO_4 Solvent B: CH_3CN			
GRADIENT:	Time (min.)	% A	%B	Flow (mL/min)
	0.01	90.0	10.0	1.000
	1.00	90.0	10.0	1.000
	8.00	5.00	95.0	1.000
	10.00	5.00	95.0	1.000
	10.10	90.0	10.0	1.000
	15.00	90.0	10.0	1.000
INJECTION VOLUME:	100.0 µL			
TBBPA RETENTION TIME:	Approximately 10.9 minutes			
PRIMARY ANALYTICAL WAVELENGTH:	286 nm			

Table 2

Measured Concentration of Tetrabromobisphenol A in Samples from a Seedling Emergence Study

Nominal Test Concentration (mg/Kg)	Sample Number (439-102)[1]	Measured Concentration (mg/Kg)[2,3]	Percent of Nominal[3] (%)	Mean Concentration (mg/Kg)	Mean Percent of Nominal (%)
0.0	MAB-1	< LOQ	--	--	--
15.0	MAS-1	14.7	97.9	--	--
100	MAS-2	94.9	94.9	--	--
5000	MAS-3	4970	99.4	--	--
Negative Control	1	< LOQ	--	--	--
20	2	15.1	75.7	16	80.0
	3	15.9	79.7		
	4	16.7	83.5		
78	5	61.5	78.8	64	82.1
	6	71.0	91.0		
	7	60.1	77.1		
313	8	211	67.3	253	80.8
	9	287	91.5		
	10	261	83.3		
1250	11	970	77.6	1059	84.7
	12	1047	83.8		
	13	1161	92.9		
5000	14	4289	85.8	4595	91.9
	15	4669	93.4		
	16	4828	96.6		

[1] MAB refers to an unfortified matrix blank. MAS refers to a fortified quality control sample.

[2] The limit of quantitation (LOQ) was defined as 10.0 mg/Kg, calculated as the product of the lowest calibration standard concentration (0.100 mg/L) and the dilution factor of the matrix blank sample (100).

[3] Results were generated using Excel 2000 in the full precision mode. Manual calculations may differ slightly.

METHOD OUTLINE FOR THE PROCESSING OF
TETRABROMOBISPHENOL A IN SOIL

Rinse 8 oz. French square bottles with methanol

↓

Transfer the requisite volume of sediment to the French square bottles. Fortify samples with the appropriate Tetrabromobisphenol A stock solution or the test substance. Unfortified sediment will serve as the matrix blank samples.

↓

Using a class A volumetric pipette, add 100 mL of acidified methanol to each sample.

↓

Sonic disrupt the samples for approximately 10 minutes at a setting of approximately 60.

↓

Allow the samples to settle and cool for approximately five minutes.

↓

Transfer an aliquot of each sample into microcentrifuge tubes and centrifuge for approximately five minutes.

↓

Volumetrically dilute the centrifuged methanol extract using the requisite volume of 50% methanol : 50% water.

↓

Transfer an aliquot of each diluted extract to an autosampler vial. Submit samples for HPLC/UV analysis.

Figure 1. A method flowchart for the analysis of TBBPA in soil samples.

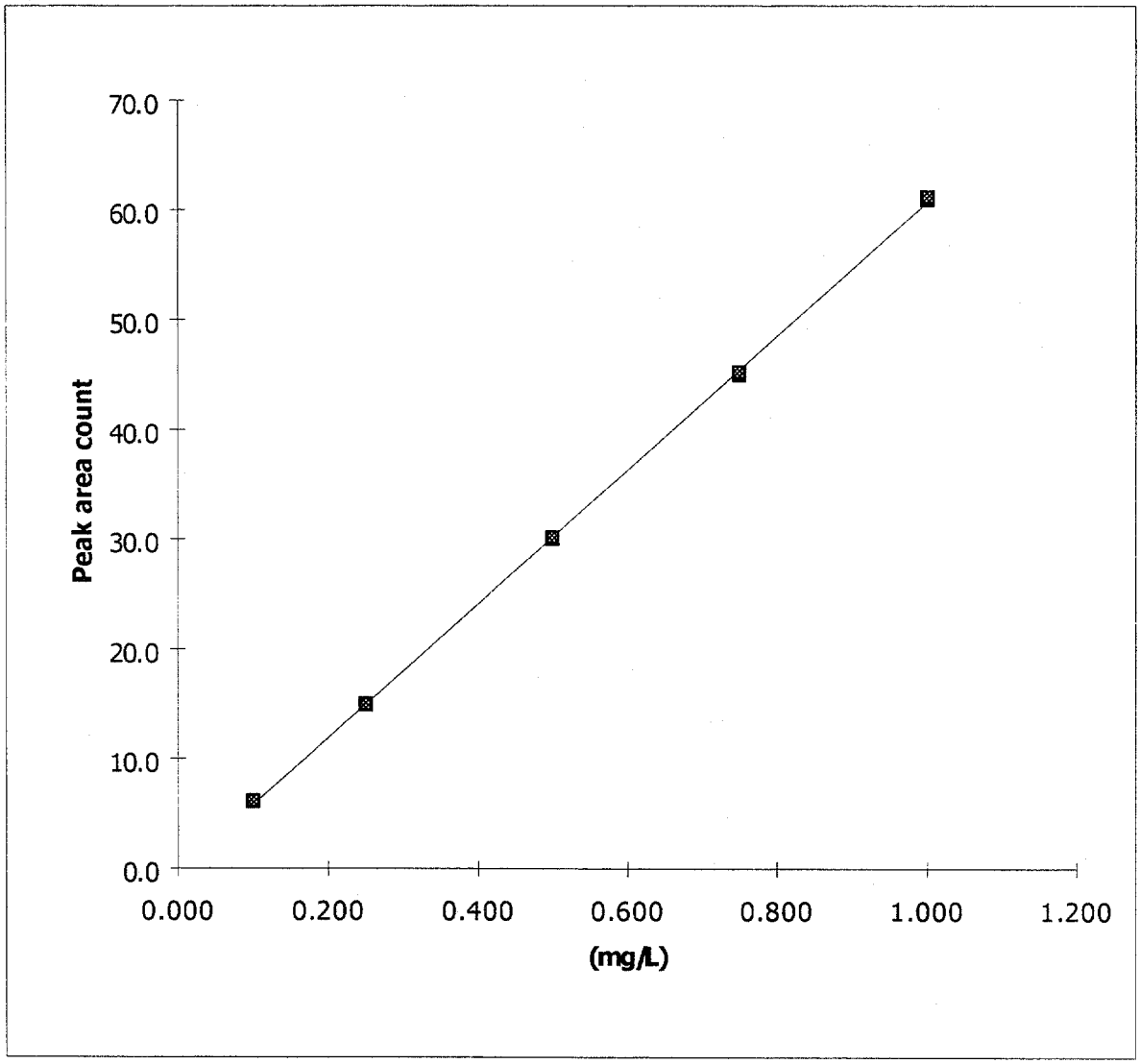


Figure 2. A representative calibration curve for TBBPA. Slope = 60.9319; Y-Intercept = -0.2275; $r^2 = 0.9998$.

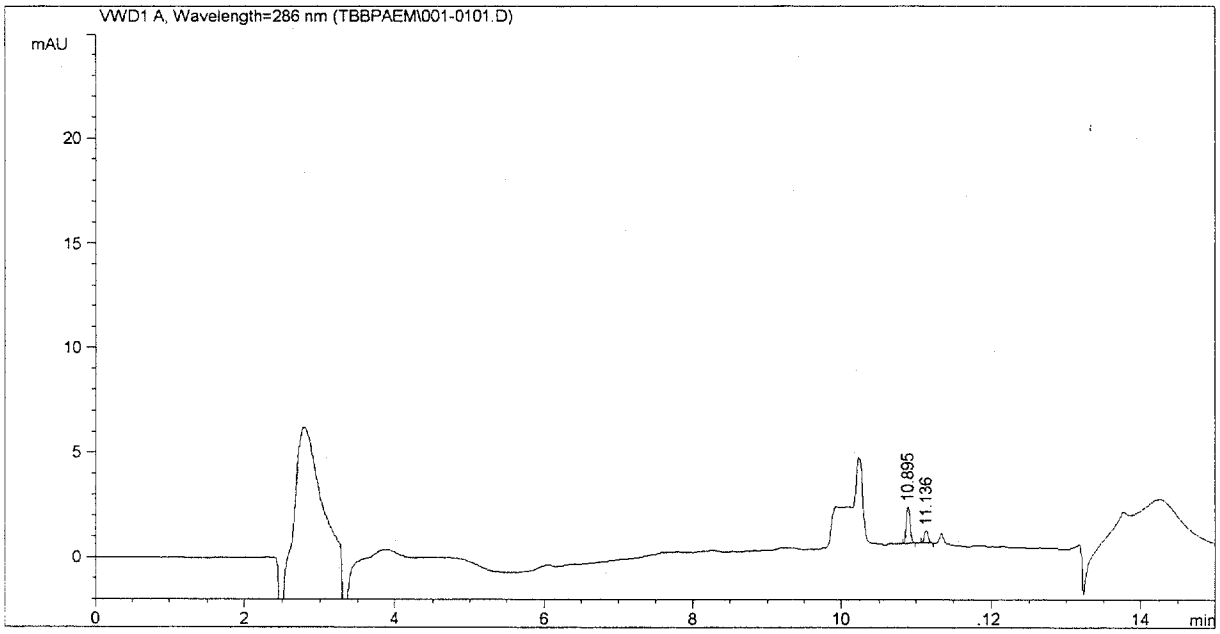


Figure 3. A representative chromatogram of a 0.100 mg/L calibration standard.

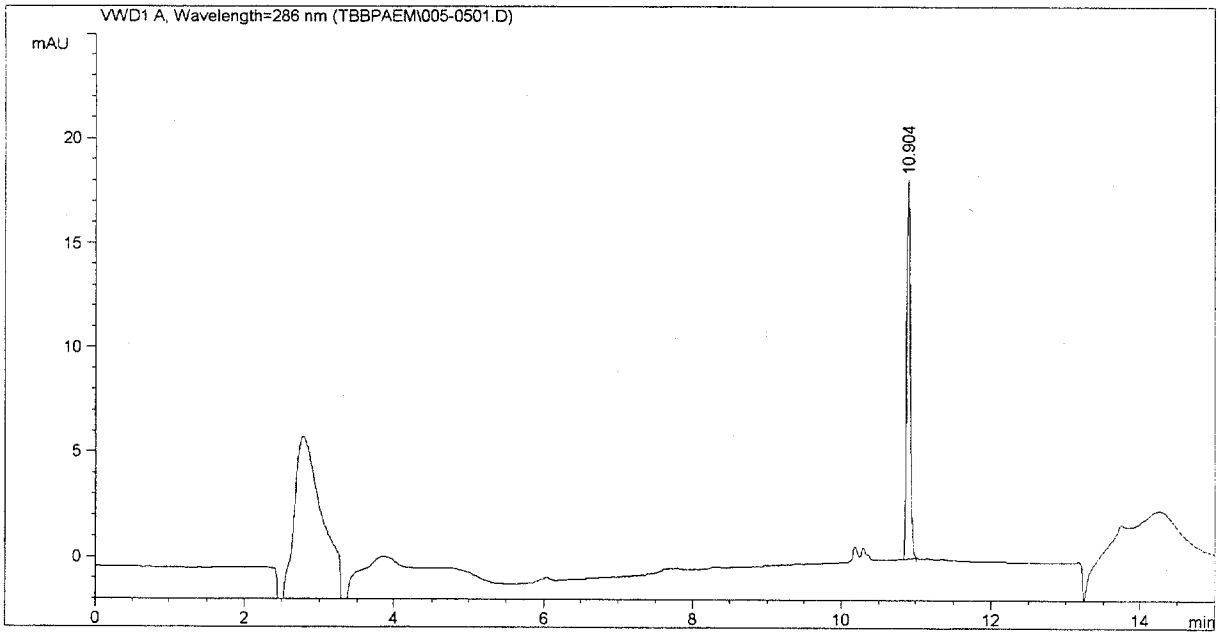


Figure 4. A representative chromatogram of a 1.00 mg/L calibration standard.

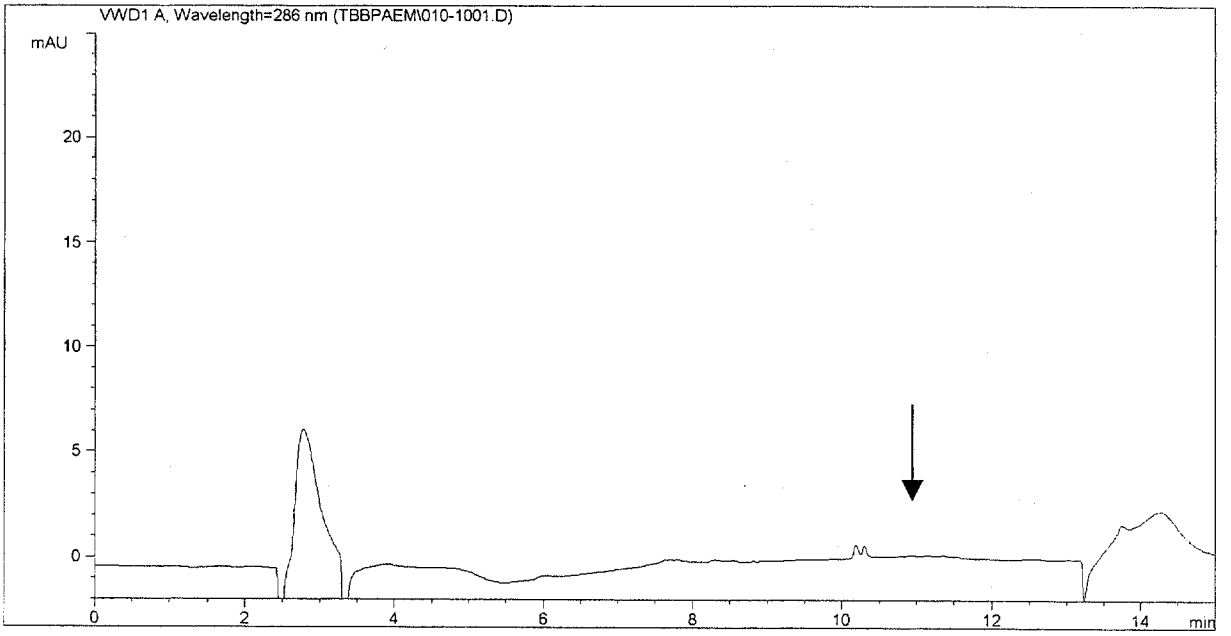


Figure 5. A representative chromatogram of a control sample (439-102-1). The arrow indicates the retention time of TBBPA.

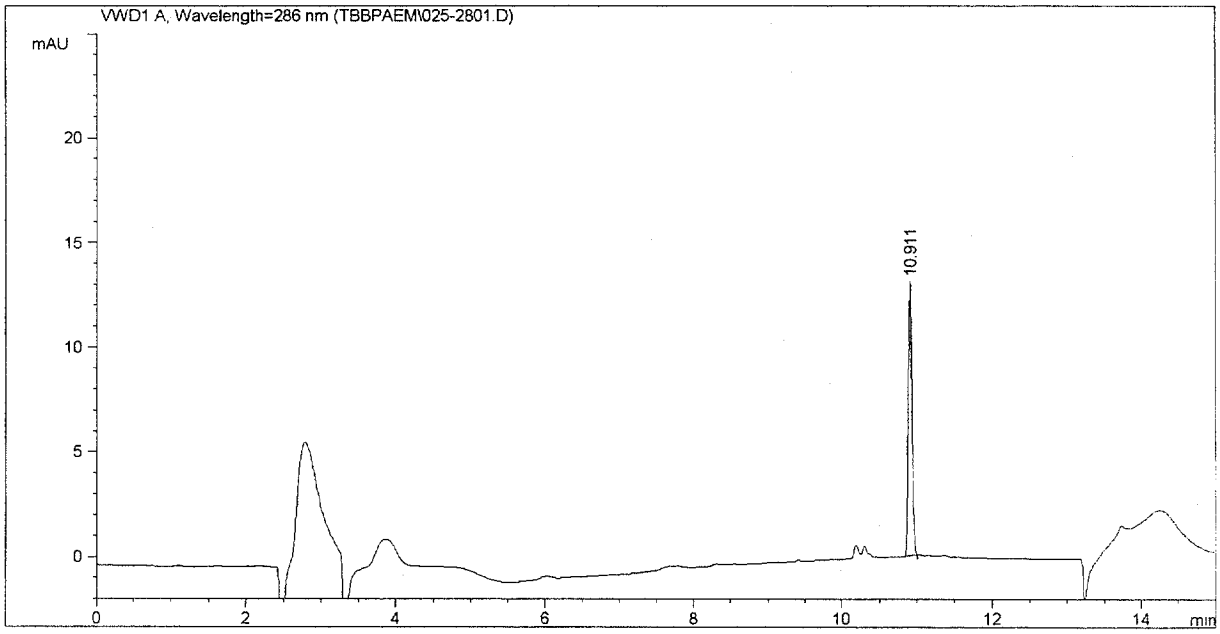


Figure 6. A representative chromatogram of a soil extract (439-102-16, 5000 mg/Kg nominal concentration).

Appendix 5

Environmental Conditions

Date	Temperature (°C)			Relative Humidity (%)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
11/14/01[1]	16	26	20	17	72	39
11/15/01	16	27	21	20	63	39
11/16/01	17	29	21	21	63	42
11/17/01[1]	16	26	20	27	63	44
11/18/01[1]	16	27	20	21	54	38
11/19/01	16	27	21	30	81	50
11/20/01	16	27	20	13	81	40
11/21/01[1]	16	27	20	12	51	33
11/22/01	16	28	20	14	47	32
11/23/01[1]	16	27	20	16	57	34
11/24/01	17	25	21	46	75	60
11/25/01	18	26	21	42	83	64
11/26/01[1]	17	27	21	31	85	59
11/27/01	17	25	20	35	75	53
11/28/01	18	26	21	47	80	66
11/29/01	18	26	21	49	81	68
11/30/01	18	26	22	54	85	70
12/01/01	17	28	21	36	83	61
12/02/01[2]	16	27	20	22	55	39
12/03/01[1]	16	32	21	19	55	37
12/04/01	16	27	21	20	57	40
12/05/01	17	29	21	17	59	40

[1] Indicates days on which all species were watered.
[2] Indicates days on which only cucumber, soybean and ryegrass were watered.

Appendix 6.1

Corn Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	8	10	*4*	9.00	1.15
20 mg/kg	10	10	10	10	*4*	10.00	0.00
78 mg/kg	10	8	9	9	*4*	9.00	0.82
313 mg/kg	10	9	10	7	*4*	9.00	1.41
1250 mg/kg	9	10	8	9	*4*	9.00	0.82
5000 mg/kg	10	10	9	10	*4*	9.75	0.50

Day 14

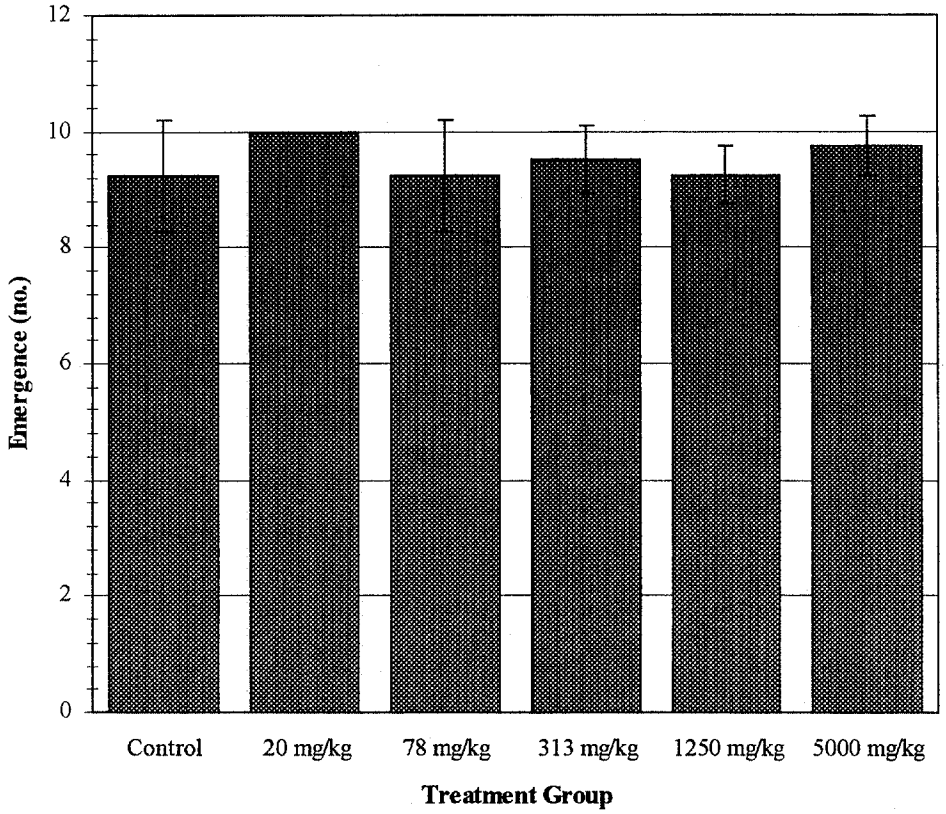
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	9	10	*4*	9.25	0.96
20 mg/kg	10	10	10	10	*4*	10.00	0.00
78 mg/kg	10	8	10	9	*4*	9.25	0.96
313 mg/kg	10	9	10	9	*4*	9.50	0.58
1250 mg/kg	9	10	9	9	*4*	9.25	0.50
5000 mg/kg	10	10	9	10	*4*	9.75	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	9	10	*4*	9.25	0.96
20 mg/kg	10	10	10	10	*4*	10.00	0.00
78 mg/kg	10	8	10	9	*4*	9.25	0.96
313 mg/kg	10	9	10	9	*4*	9.50	0.58
1250 mg/kg	9	10	9	9	*4*	9.25	0.50
5000 mg/kg	10	10	9	10	*4*	9.75	0.50

Appendix 6.2

Mean Corn Emergence on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

Appendix 6.3

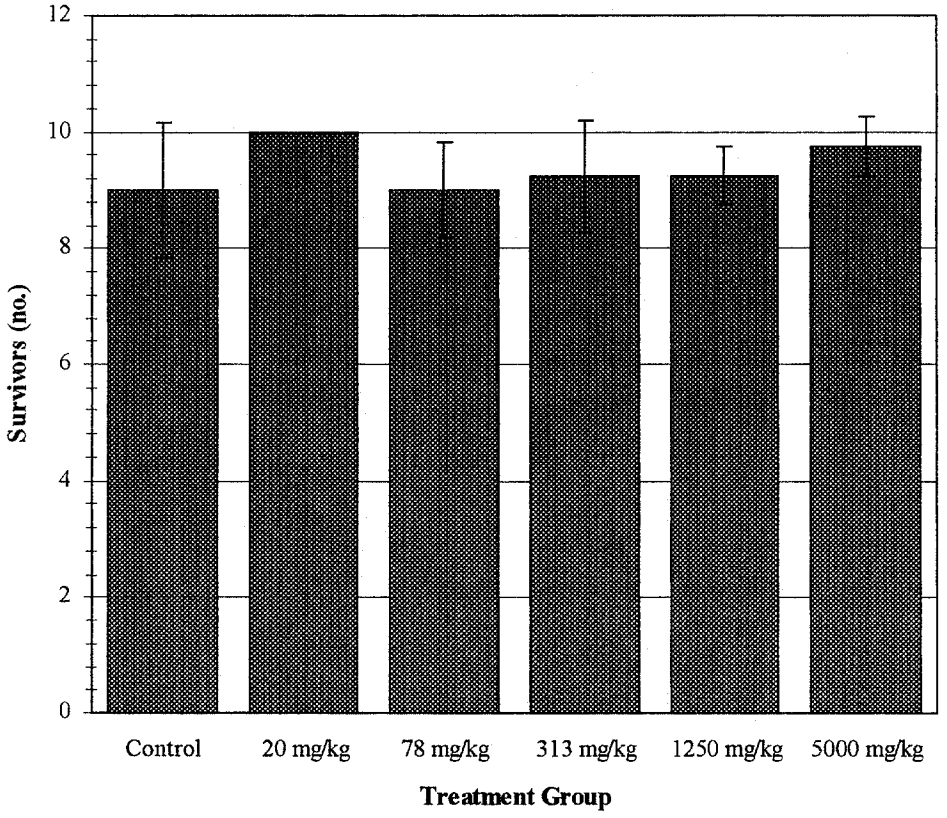
Corn 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	8	10	*4*	9.00	1.15
20 mg/kg	10	10	10	10	*4*	10.00	0.00
78 mg/kg	10	8	9	9	*4*	9.00	0.82
313 mg/kg	10	9	10	8	*4*	9.25	0.96
1250 mg/kg	9	10	9	9	*4*	9.25	0.50
5000 mg/kg	10	10	9	10	*4*	9.75	0.50

Appendix 6.4

Mean Corn 21-Day Survival



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

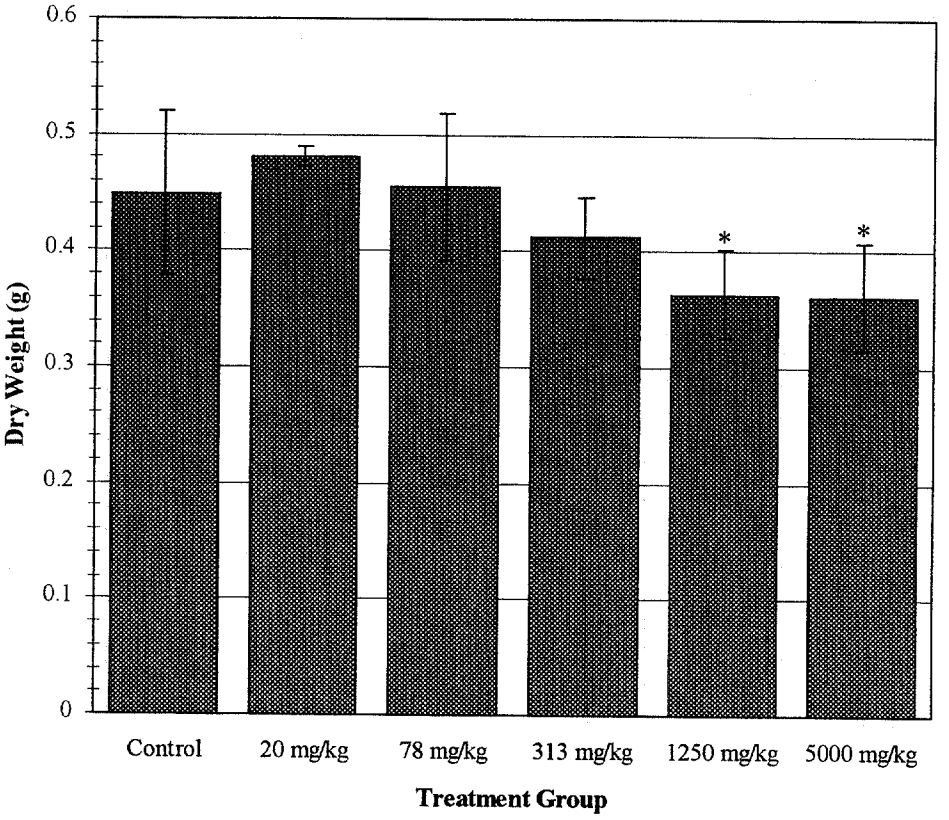
Appendix 6.5

Corn Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.49	0.49	0.47	0.34	*4*	0.45	0.071
20 mg/kg	0.48	0.49	0.48	0.47	*4*	0.48	0.008
78 mg/kg	0.42	0.55	0.43	0.41	*4*	0.45	0.063
313 mg/kg	0.43	0.42	0.43	0.36	*4*	0.41	0.036
1250 mg/kg	0.32	0.36	0.37	0.41	*4*	0.36	0.038
5000 mg/kg	0.35	0.42	0.31	0.36	*4*	0.36	0.046

Appendix 6.6

Mean Corn Dry Weight



* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Appendix 6.7

Corn Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	42	41	33	48	42	38	37	45	36	48	*10*	41.0	5.06
	B	.	.	46	52	40	43	26	32	51	45	*8*	41.9	9.00
	C	.	.	42	40	44	46	37	46	40	48	*8*	42.9	3.76
	D	35	39	40	18	33	34	31	32	35	32	*10*	32.9	6.01
20 mg/kg	A	39	50	44	49	44	43	38	42	49	32	*10*	43.0	5.64
	B	48	42	42	37	48	44	45	39	52	41	*10*	43.8	4.57
	C	46	42	38	44	50	34	45	51	50	42	*10*	44.2	5.47
	D	42	44	43	47	45	41	48	50	44	51	*10*	45.5	3.37
78 mg/kg	A	37	39	35	32	45	38	38	41	40	41	*10*	38.6	3.57
	B	40	40	50	52	54	61	29	44	.	.	*8*	46.3	10.04
	C	43	46	44	47	44	39	35	37	38	.	*9*	41.4	4.28
	D	.	42	33	42	41	43	42	43	41	30	*9*	39.7	4.74
313 mg/kg	A	44	29	42	38	42	46	42	34	38	47	*10*	40.2	5.55
	B	.	34	49	41	42	35	40	33	45	31	*9*	38.9	6.03
	C	49	51	46	44	37	42	40	39	30	41	*10*	41.9	6.08
	D	.	.	41	37	42	39	34	23	27	43	*8*	35.8	7.30
1250 mg/kg	A	.	39	39	33	35	29	26	30	35	34	*9*	33.3	4.39
	B	33	38	46	38	39	43	34	35	35	33	*10*	37.4	4.35
	C	.	36	37	38	36	29	36	33	47	28	*9*	35.6	5.55
	D	.	33	37	42	44	28	38	33	38	41	*9*	37.1	5.06
5000 mg/kg	A	18	35	43	36	40	47	39	42	30	26	*10*	35.6	8.76
	B	39	41	35	43	52	41	37	41	35	19	*10*	38.3	8.35
	C	.	33	35	31	31	32	38	36	35	42	*9*	34.8	3.60
	D	38	32	16	45	42	27	37	37	39	32	*10*	34.5	8.32

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

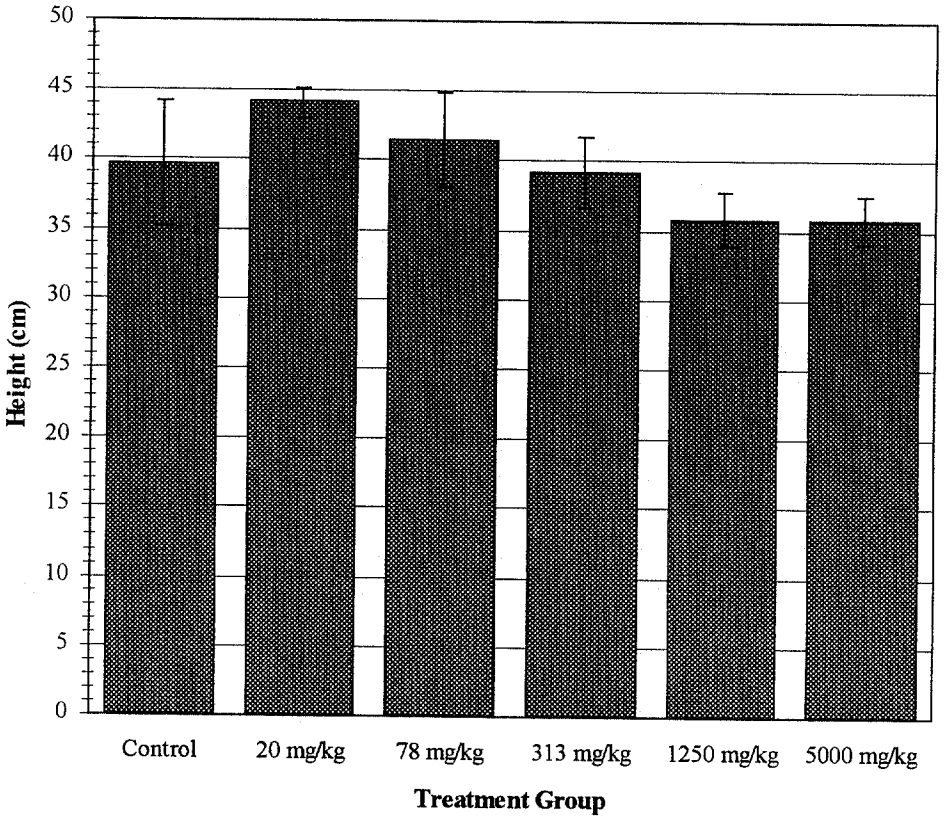
Appendix 6.8

Corn Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	41.0	41.9	42.9	32.9	*4*	39.7	4.57
20 mg/kg	43.0	43.8	44.2	45.5	*4*	44.1	1.04
78 mg/kg	38.6	46.3	41.4	39.7	*4*	41.5	3.38
313 mg/kg	40.2	38.9	41.9	35.8	*4*	39.2	2.60
1250 mg/kg	33.3	37.4	35.6	37.1	*4*	35.9	1.86
5000 mg/kg	35.6	38.3	34.8	34.5	*4*	35.8	1.73

Appendix 6.9

Mean Corn Height on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$)

Appendix 6.10

Corn Seedling Condition, Day 21

Treatment Group T	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
20 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
78 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	.	*8*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	100.-	*10*	10	31.6
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
313 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
1250 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
5000 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 7.1

Cucumber Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	7	10	9	*4*	8.50	1.29
20 mg/kg	7	9	7	10	*4*	8.25	1.50
78 mg/kg	7	9	9	7	*4*	8.00	1.15
313 mg/kg	9	8	10	6	*4*	8.25	1.71
1250 mg/kg	9	10	7	10	*4*	9.00	1.41
5000 mg/kg	7	9	7	4	*4*	6.75	2.06

Day 14

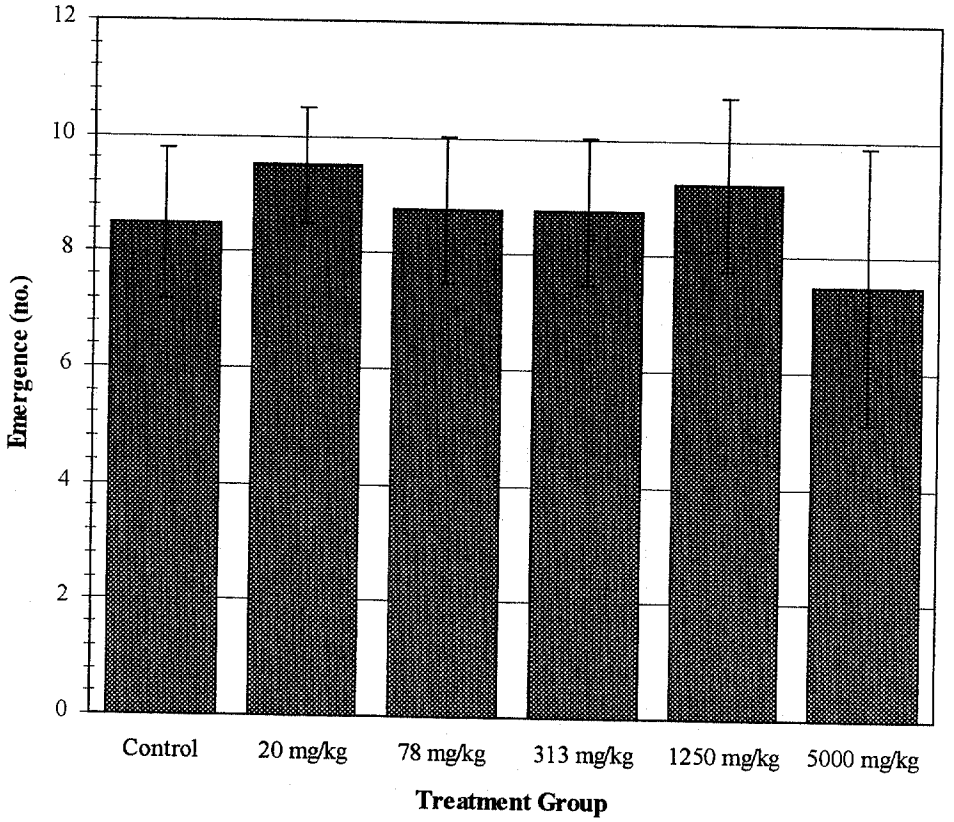
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	7	10	9	*4*	8.50	1.29
20 mg/kg	8	10	10	10	*4*	9.50	1.00
78 mg/kg	7	9	10	8	*4*	8.50	1.29
313 mg/kg	9	9	10	7	*4*	8.75	1.26
1250 mg/kg	9	10	7	10	*4*	9.00	1.41
5000 mg/kg	9	9	8	4	*4*	7.50	2.38

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	7	10	9	*4*	8.50	1.29
20 mg/kg	8	10	10	10	*4*	9.50	1.00
78 mg/kg	7	9	10	9	*4*	8.75	1.26
313 mg/kg	9	9	10	7	*4*	8.75	1.26
1250 mg/kg	10	10	7	10	*4*	9.25	1.50
5000 mg/kg	9	9	8	4	*4*	7.50	2.38

Appendix 7.2

Mean Cucumber Emergence on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$)

Appendix 7.3

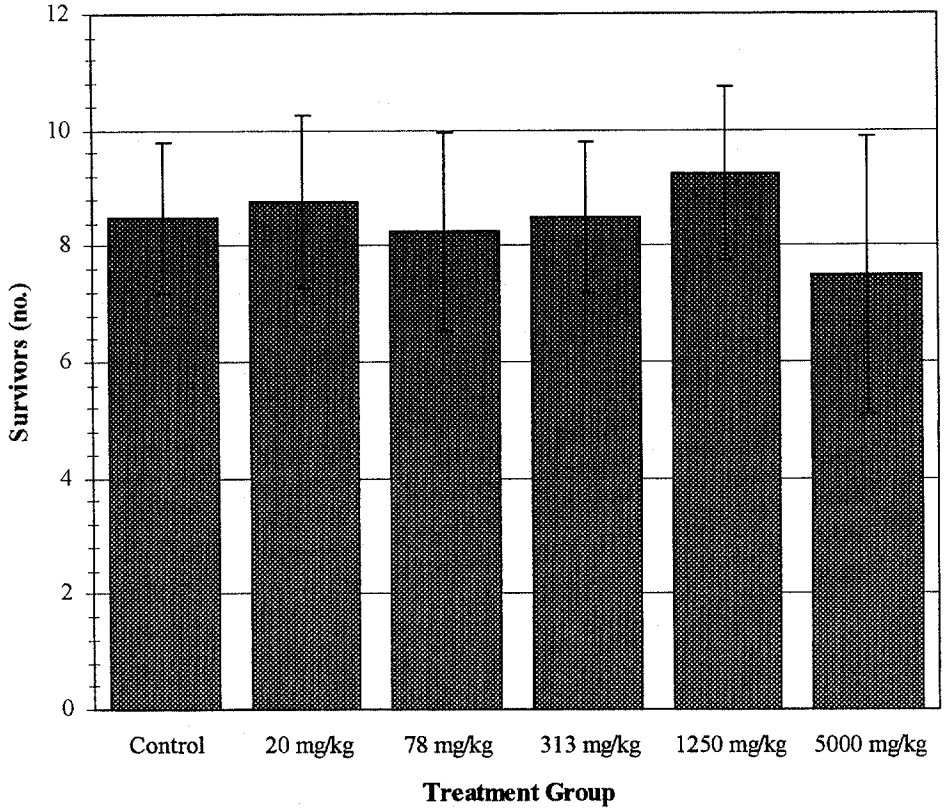
Cucumber 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	7	10	9	*4*	8.50	1.29
20 mg/kg	8	10	7	10	*4*	8.75	1.50
78 mg/kg	6	9	10	8	*4*	8.25	1.71
313 mg/kg	8	9	10	7	*4*	8.50	1.29
1250 mg/kg	10	10	7	10	*4*	9.25	1.50
5000 mg/kg	9	9	8	4	*4*	7.50	2.38

Appendix 7.4

Mean Cucumber 21-Day Survival



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

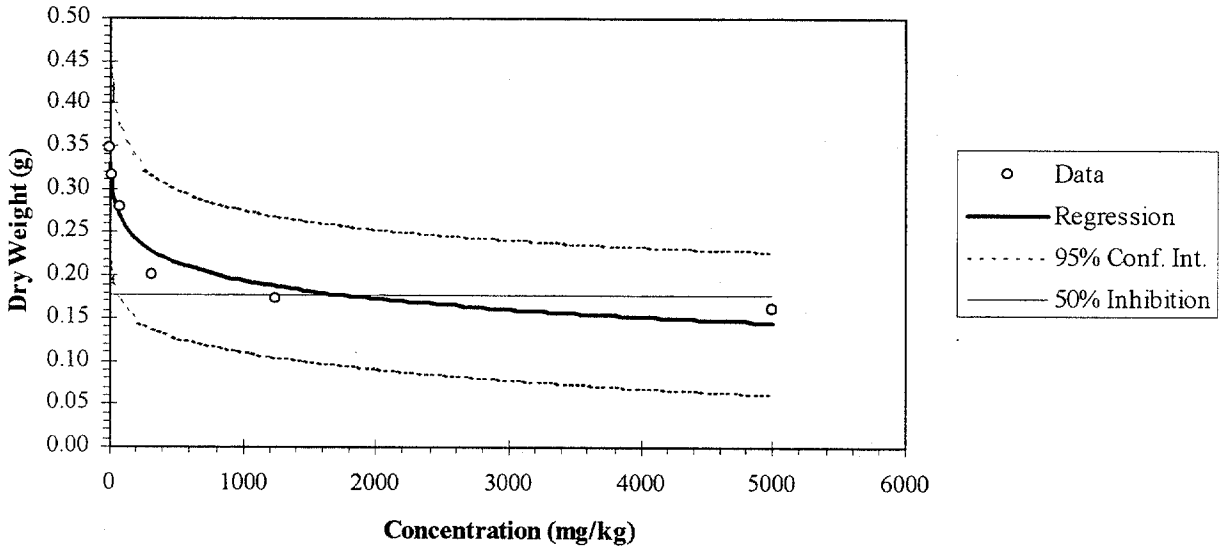
Appendix 7.5

Cucumber Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.38	0.38	0.32	0.31	*4*	0.35	0.037
20 mg/kg	0.33	0.30	0.31	0.32	*4*	0.32	0.014
78 mg/kg	0.32	0.34	0.25	0.22	*4*	0.28	0.056
313 mg/kg	0.25	0.19	0.21	0.16	*4*	0.20	0.035
1250 mg/kg	0.16	0.18	0.18	0.17	*4*	0.17	0.011
5000 mg/kg	0.13	0.18	0.16	0.17	*4*	0.16	0.020

Appendix 7.6

Mean Cucumber Dry Weight



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
72.6440	1.73700	3038.79	0.3567	2.0193	0.92881

EC_{50}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
1672.25	194.223	14394.61	0.3567	2.0193	0.92881

Appendix 7.7

Cucumber Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	9	9	10	9	13	10	16	10	*8*	10.8	2.49
	B	.	.	.	9	11	9	12	13	9	9	*7*	10.3	1.70
	C	10	12	13	10	3	6	13	8	10	10	*10*	9.5	3.14
	D	.	10	10	8	8	10	8	5	8	7	*9*	8.2	1.64
20 mg/kg	A	.	.	8	11	7	10	8	7	9	9	*8*	8.6	1.41
	B	2	8	8	9	8	10	13	12	13	11	*10*	9.4	3.27
	C	.	.	.	10	12	8	11	9	10	8	*7*	9.7	1.50
	D	8	10	9	11	10	9	11	9	11	10	*10*	9.8	1.03
78 mg/kg	A	.	.	.	.	7	5	7	7	8	4	*6*	6.3	1.51
	B	.	8	12	5	10	14	9	11	8	9	*9*	9.6	2.60
	C	9	8	3	10	8	8	12	8	8	9	*10*	8.3	2.26
	D	.	.	3	5	6	7	6	7	6	7	*8*	5.9	1.36
313 mg/kg	A	.	.	5	7	5	10	6	7	9	5	*8*	6.8	1.91
	B	.	7	7	9	7	7	5	7	8	8	*9*	7.2	1.09
	C	5	8	9	7	5	10	7	7	8	7	*10*	7.3	1.57
	D	.	.	.	3	7	3	5	4	5	6	*7*	4.7	1.50
1250 mg/kg	A	4	8	7	5	4	6	5	4	7	1	*10*	5.1	2.02
	B	6	7	8	6	8	5	5	7	5	3	*10*	6.0	1.56
	C	.	.	.	6	5	6	4	2	5	7	*7*	5.0	1.63
	D	7	5	5	6	5	6	5	4	4	5	*10*	5.2	0.92
5000 mg/kg	A	.	5	5	6	5	7	2	1	6	3	*9*	4.4	2.01
	B	.	4	6	6	6	4	5	7	5	6	*9*	5.4	1.01
	C	.	4	4	5	7	4	4	4	4	.	*8*	4.5	1.07
	D	.	.	.	.	.	.	4	4	4	5	*4*	4.3	0.50

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

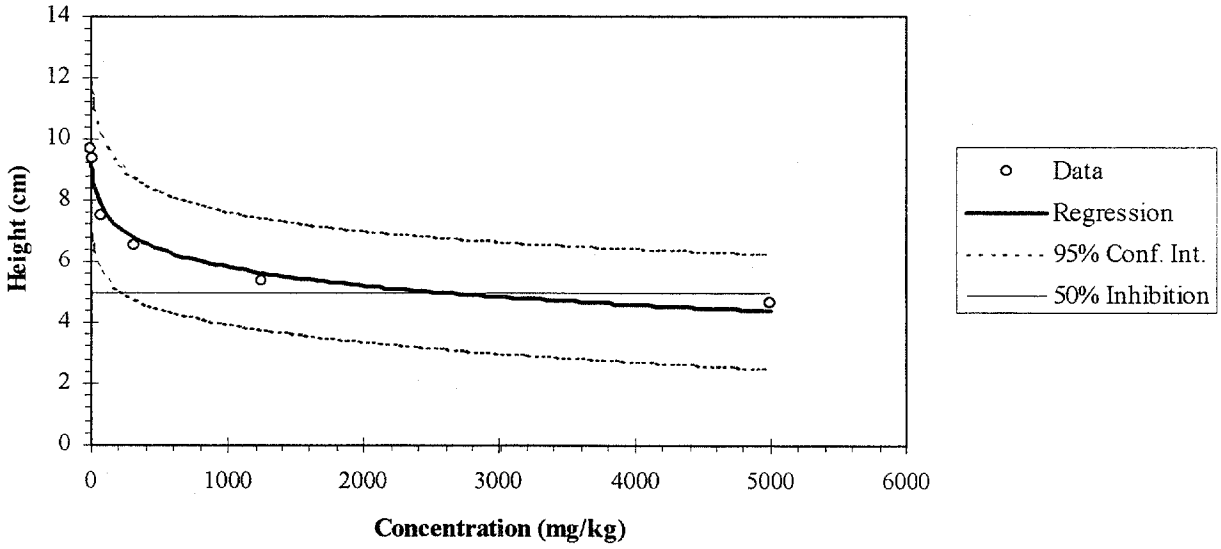
Appendix 7.8

Cucumber Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10.8	10.3	9.5	8.2	*4*	9.7	1.11
20 mg/kg	8.6	9.4	9.7	9.8	*4*	9.4	0.53
78 mg/kg	6.3	9.6	8.3	5.9	*4*	7.5	1.72
313 mg/kg	6.8	7.2	7.3	4.7	*4*	6.5	1.21
1250 mg/kg	5.1	6.0	5.0	5.2	*4*	5.3	0.46
5000 mg/kg	4.4	5.4	4.5	4.3	*4*	4.7	0.53

Appendix 7.9

Mean Cucumber Height on Day 21



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
131.341	8.02971	2148.33	9.9218	1.9229	0.94272

EC_{50}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
2602.56	506.407	13375.19	9.9218	1.9229	0.94272

Appendix 7.10

Cucumber Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
20 mg/kg	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	30.LC	0.-	30.LC	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	6	12.6
	C	100.-	100.-	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	30	48.3
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
78 mg/kg	A	.	.	.	100.-	0.-	0.-	0.-	0.-	0.-	50.LC	*7*	21	39.3
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
313 mg/kg	A	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	.	.	0.-	0.-	30.LC	0.-	0.-	0.-	0.-	*7*	4	11.3
1250 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	80.N	*10*	8	25.3
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
5000 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	50.LC	80.N	0.-	0.-	*9*	14	29.6
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	*8*	0	0.0
	D	.	.	.	.	.	.	0.-	0.-	0.-	0.-	*4*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity. LC – Leaf Curl, N - Necrosis

Appendix 8.1

Onion Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	8	10	7	9	*4*	8.50	1.29
20 mg/kg	10	8	8	10	*4*	9.00	1.15
78 mg/kg	9	10	9	9	*4*	9.25	0.50
313 mg/kg	10	10	9	9	*4*	9.50	0.58
1250 mg/kg	8	10	9	9	*4*	9.00	0.82
5000 mg/kg	9	8	7	9	*4*	8.25	0.96

Day 14

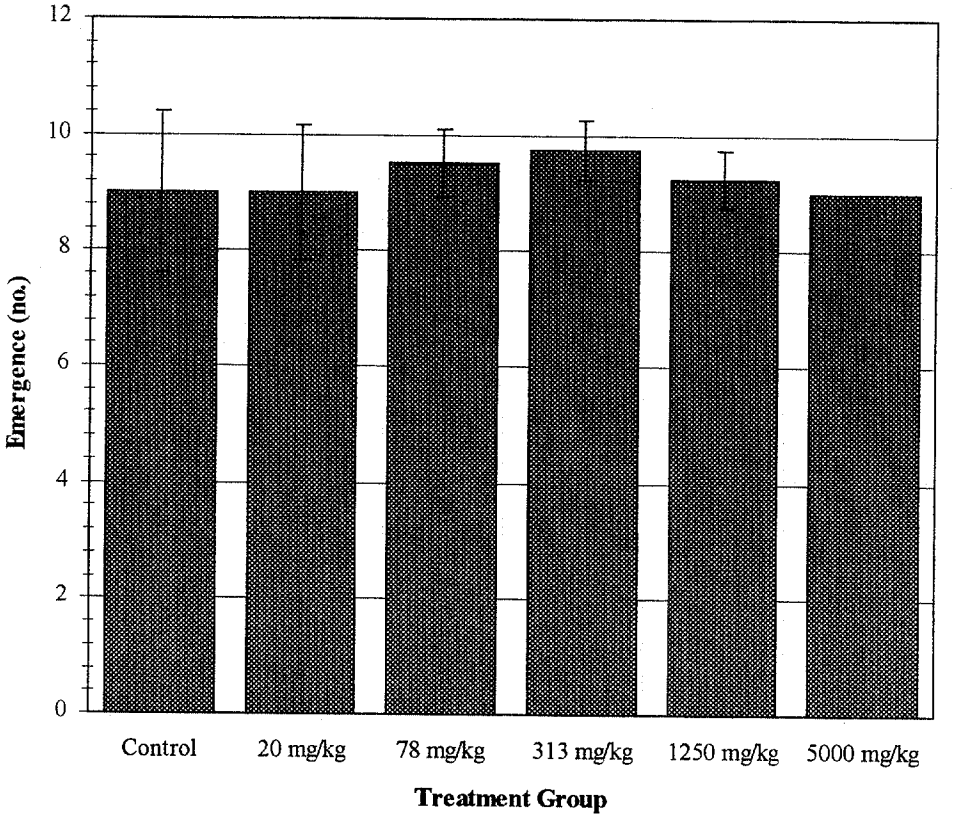
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	7	10	*4*	9.00	1.41
20 mg/kg	10	8	8	10	*4*	9.00	1.55
78 mg/kg	10	10	9	9	*4*	9.50	0.58
313 mg/kg	10	10	10	9	*4*	9.75	0.50
1250 mg/kg	9	10	9	9	*4*	9.25	0.50
5000 mg/kg	9	9	9	9	*4*	9.00	0.00

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	7	10	*4*	9.00	1.41
20 mg/kg	10	8	8	10	*4*	9.00	1.55
78 mg/kg	10	10	9	9	*4*	9.50	0.58
313 mg/kg	10	10	10	9	*4*	9.75	0.50
1250 mg/kg	9	10	9	9	*4*	9.25	0.50
5000 mg/kg	9	9	9	9	*4*	9.00	0.00

Appendix 8.2

Mean Onion Emergence on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

Appendix 8.3

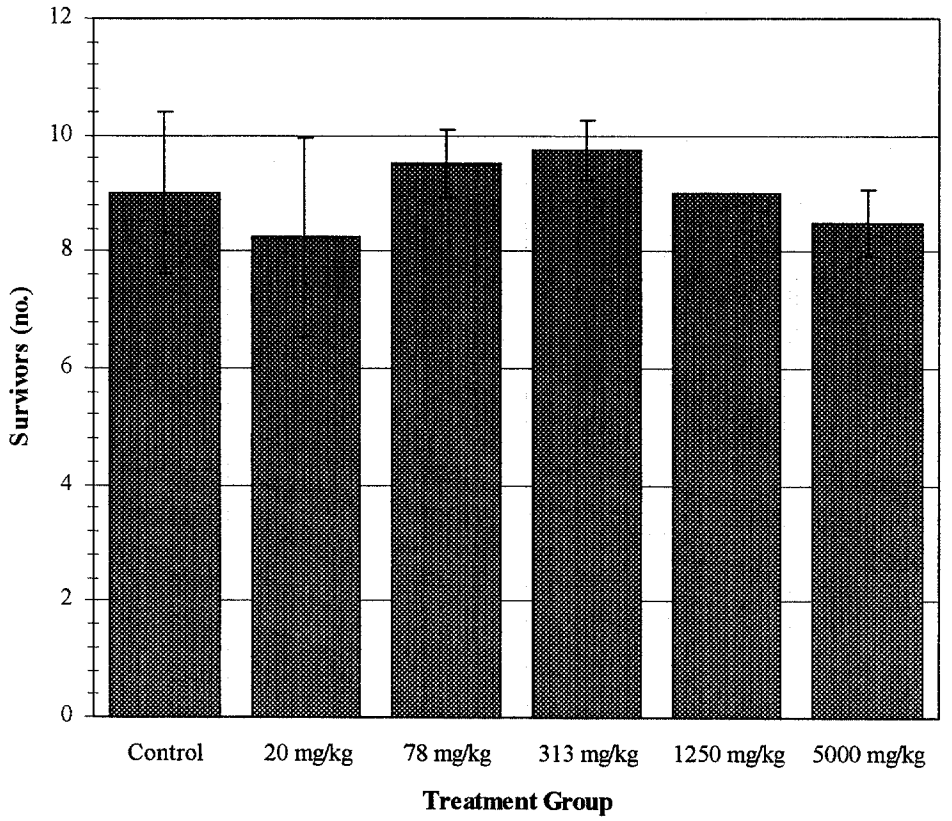
Onion 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	7	10	*4*	9.00	1.41
20 mg/kg	9	8	6	10	*4*	8.25	1.71
78 mg/kg	10	10	9	9	*4*	9.50	0.58
313 mg/kg	10	10	10	9	*4*	9.75	0.50
1250 mg/kg	9	9	9	9	*4*	9.00	0.00
5000 mg/kg	9	9	8	8	*4*	8.50	0.58

Appendix 8.4

Mean Onion 21-Day Survival



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

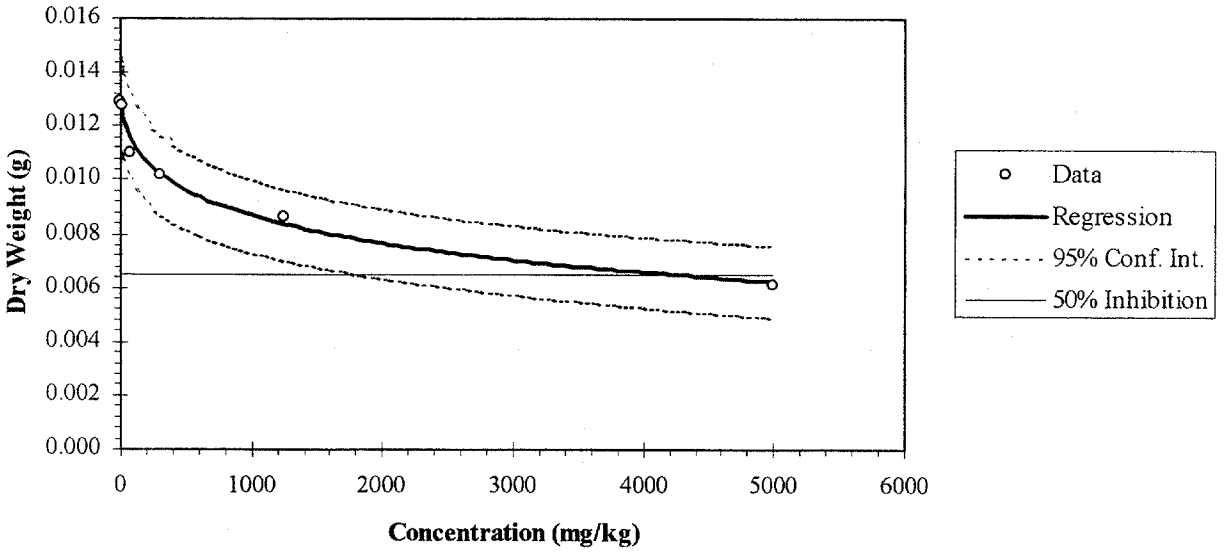
Appendix 8.5

Onion Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.012	0.016	0.011	0.012	*4*	0.013	0.0021
20 mg/kg	0.013	0.016	0.013	0.008	*4*	0.013	0.0034
78 mg/kg	0.014	0.010	0.010	0.010	*4*	0.011	0.0020
313 mg/kg	0.014	0.010	0.009	0.008	*4*	0.010	0.0027
1250 mg/kg	0.011	0.007	0.009	0.008	*4*	0.009	0.0019
5000 mg/kg	0.008	0.006	0.006	0.005	*4*	0.006	0.0012

Appendix 8.6

Mean Onion Dry Weight



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
459.727	150.349	1405.72	0.0130	1.4341	0.98599

EC_{50}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
4263.83	2235.12	8135.80	0.0130	1.4341	0.98599

Appendix 8.7

Onion Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	2	7	8	7	13	4	5	11	8	*9*	7.2	3.38
	B	9	9	9	7	8	6	7	14	8	7	*10*	8.4	2.22
	C	.	.	.	7	8	8	6	6	4	13	*7*	7.4	2.82
	D	9	6	8	6	9	7	6	5	6	3	*10*	6.5	1.84
20 mg/kg	A	.	9	9	8	7	10	8	7	12	11	*9*	9.0	1.73
	B	.	.	5	10	10	6	7	7	8	7	*8*	7.5	1.77
	C	.	.	.	.	7	7	6	7	7	8	*6*	7.0	0.63
	D	6	8	6	8	7	8	9	13	8	7	*10*	8.0	2.00
78 mg/kg	A	9	9	9	9	8	9	8	10	9	1	*10*	8.1	2.56
	B	8	9	7	7	8	7	7	9	9	8	*10*	7.9	0.88
	C	.	7	8	4	7	8	7	7	8	9	*9*	7.2	1.39
	D	.	7	7	8	9	6	7	3	11	7	*9*	7.2	2.17
313 mg/kg	A	7	6	7	6	3	7	6	7	7	8	*10*	6.4	1.35
	B	8	9	8	8	7	6	13	8	6	6	*10*	7.9	2.08
	C	7	4	7	8	7	8	9	8	7	8	*10*	7.3	1.34
	D	.	8	6	8	3	7	7	13	6	7	*9*	7.2	2.64
1250 mg/kg	A	.	9	4	6	8	5	7	7	6	2	*9*	6.0	2.12
	B	.	2	8	3	6	6	2	7	10	7	*9*	5.7	2.78
	C	.	6	7	6	4	3	6	5	6	7	*9*	5.6	1.33
	D	.	8	2	10	6	6	6	4	11	3	*9*	6.2	3.03
5000 mg/kg	A	.	6	5	10	4	6	3	4	5	5	*9*	5.3	2.00
	B	.	4	5	5	4	4	4	5	6	5	*9*	4.7	0.71
	C	.	.	4	5	5	5	5	6	3	6	*8*	4.9	0.99
	D	.	.	2	3	4	9	4	7	6	4	*8*	4.9	2.30

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

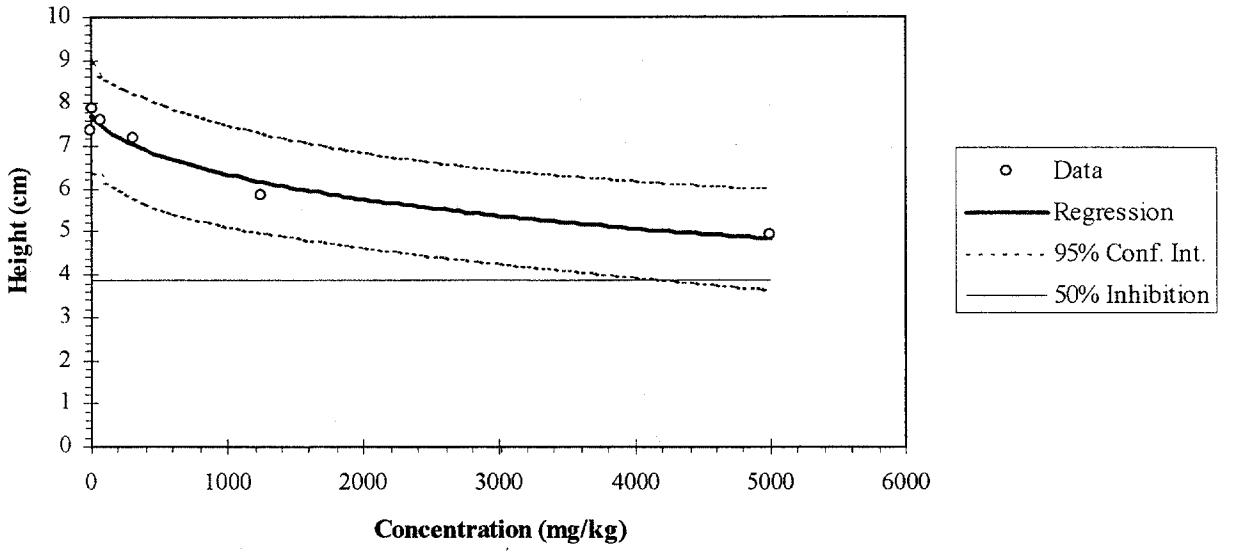
Appendix 8.8

Onion Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	7.2	8.4	7.4	6.5	*4*	7.4	0.78
20 mg/kg	9.0	7.5	7.0	8.0	*4*	7.9	0.85
78 mg/kg	8.1	7.9	7.2	7.2	*4*	7.6	0.46
313 mg/kg	6.4	7.9	7.3	7.2	*4*	7.2	0.62
1250 mg/kg	6.0	5.7	5.6	6.2	*4*	5.9	0.31
5000 mg/kg	5.3	4.7	4.9	4.9	*4*	4.9	0.28

Appendix 8.9

Mean Onion Height on Day 21



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
1947.60	668.344	5675.45	7.7132	1.1634	0.95034

Appendix 8.10

Onion Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
20 mg/kg	A	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	10	31.6
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	.	100.-	100.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	25	46.3
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
78 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
313 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
1250 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	10	31.6
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
5000 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
	D	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 9.1

Ryegrass Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	9	9	*4*	9.00	0.82
20 mg/kg	7	10	9	9	*4*	8.75	1.26
78 mg/kg	10	9	10	8	*4*	9.25	0.96
313 mg/kg	8	8	10	9	*4*	8.75	0.96
1250 mg/kg	8	6	8	10	*4*	8.00	1.63
5000 mg/kg	10	8	8	5	*4*	7.75	2.06

Day 14

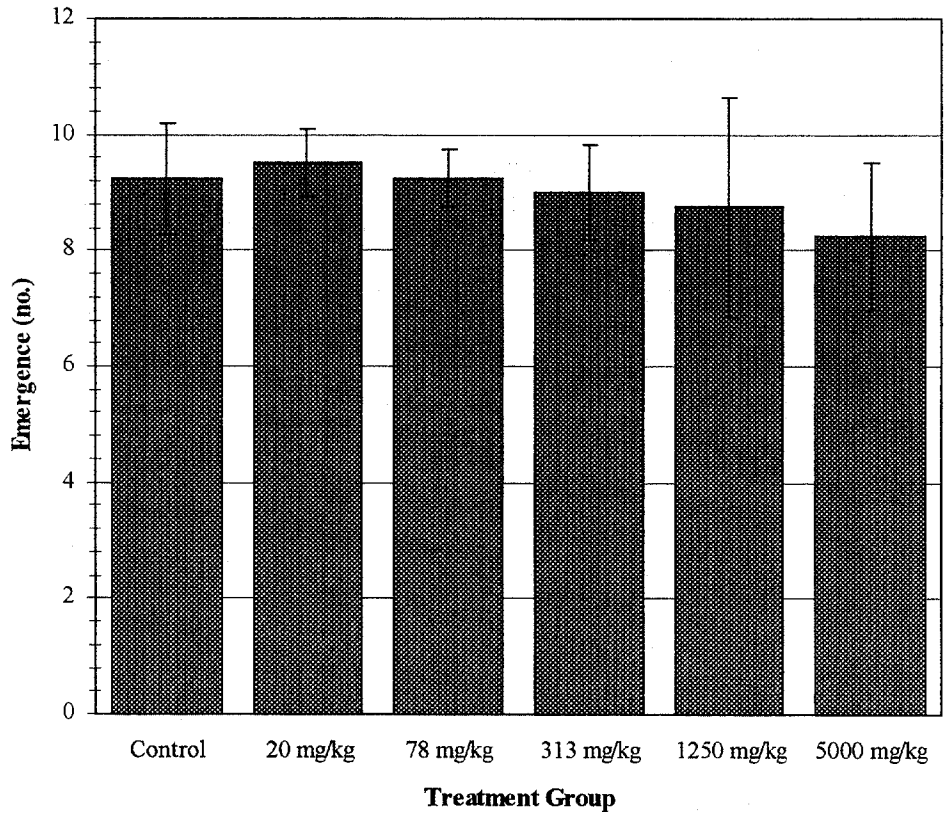
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	9	10	*4*	9.25	0.96
20 mg/kg	9	10	9	10	*4*	9.50	0.58
78 mg/kg	8	9	10	9	*4*	9.00	0.82
313 mg/kg	8	9	10	9	*4*	9.00	0.82
1250 mg/kg	9	6	9	10	*4*	8.50	1.73
5000 mg/kg	10	8	8	7	*4*	8.25	1.26

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	9	10	*4*	9.25	0.96
20 mg/kg	9	10	9	10	*4*	9.50	0.58
78 mg/kg	9	9	10	9	*4*	9.25	0.50
313 mg/kg	9	8	10	9	*4*	9.00	0.82
1250 mg/kg	9	6	10	10	*4*	8.75	1.89
5000 mg/kg	10	8	8	7	*4*	8.25	1.26

Appendix 9.2

Mean Ryegrass Emergence on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

Appendix 9.3

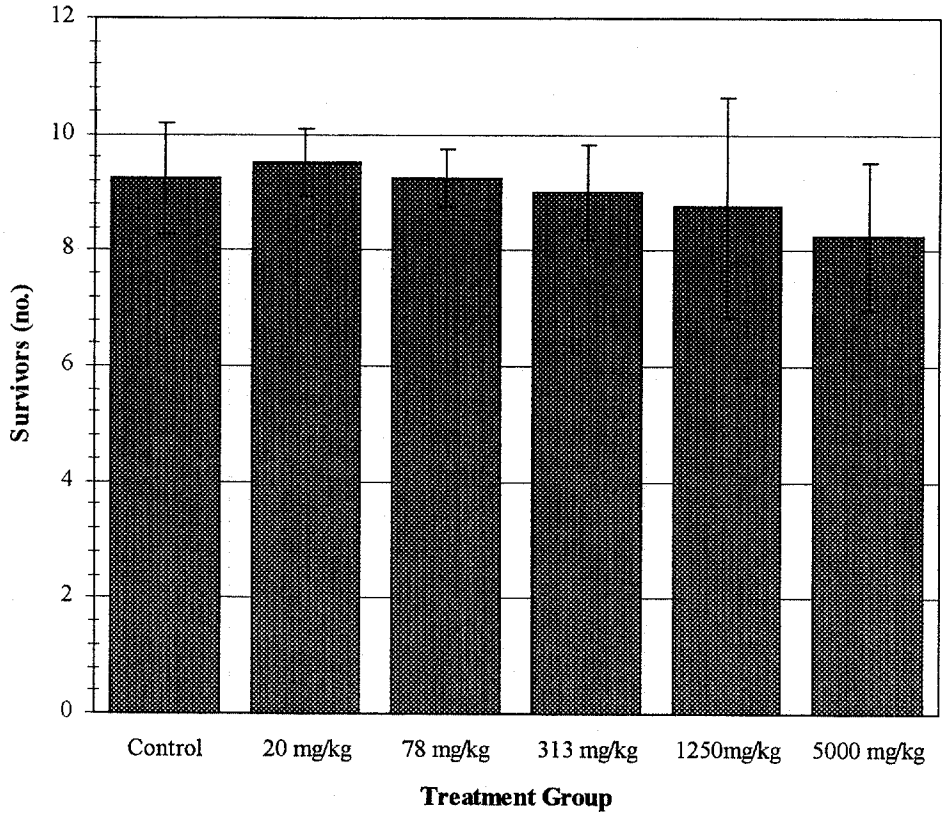
Ryegrass 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	8	9	10	*4*	9.25	0.96
20 mg/kg	9	10	9	10	*4*	9.50	0.58
78 mg/kg	9	9	10	9	*4*	9.25	0.50
313 mg/kg	9	8	10	9	*4*	9.00	0.82
1250 mg/kg	9	6	10	10	*4*	8.75	1.89
5000 mg/kg	10	8	8	7	*4*	8.25	1.26

Appendix 9.4

Mean Ryegrass 21-Day Survival



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$)

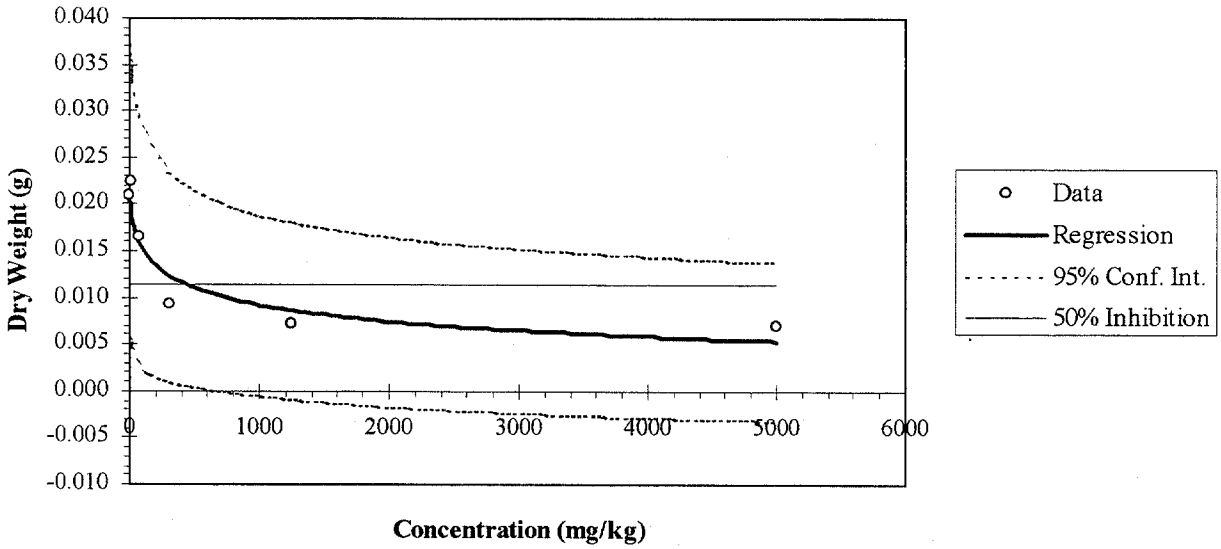
Appendix 9.5

Ryegrass Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.020	0.023	0.020	0.021	*4*	0.021	0.0012
20 mg/kg	0.030	0.023	0.020	0.017	*4*	0.023	0.0056
78 mg/kg	0.016	0.017	0.025	0.009	*4*	0.017	0.0066
313 mg/kg	0.004	0.009	0.014	0.010	*4*	0.009	0.0039
1250 mg/kg	0.007	0.003	0.010	0.009	*4*	0.007	0.0030
5000 mg/kg	0.006	0.009	0.010	0.003	*4*	0.007	0.0032

Appendix 9.6

Mean Ryegrass Dry Weight



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
48.5177	0.20188	11660.04	0.0226	1.4469	0.84141

EC_{50}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
458.987	16.2892	12930.04	0.0226	1.4469	0.84141

Appendix 9.7

Ryegrass Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	15	16	14	14	17	17	15	17	15	2	*10*	14.2	4.44
	B	.	.	16	7	16	16	22	16	16	15	*8*	15.5	4.07
	C	.	21	22	15	13	17	16	18	15	17	*9*	17.1	2.89
	D	18	18	12	9	12	19	12	16	14	16	*10*	14.6	3.31
20 mg/kg	A	.	15	24	8	17	19	14	18	23	18	*9*	17.3	4.80
	B	19	20	19	16	12	20	16	19	17	13	*10*	17.1	2.85
	C	.	15	7	14	13	17	17	14	16	20	*9*	14.8	3.60
	D	13	15	18	14	8	6	17	13	18	13	*10*	13.5	3.98
78 mg/kg	A	.	8	12	10	8	15	17	13	18	11	*9*	12.4	3.64
	B	.	10	14	17	15	19	14	15	10	7	*9*	13.4	3.78
	C	17	12	21	19	12	15	10	9	15	18	*10*	14.8	3.99
	D	.	9	13	12	10	10	5	18	11	12	*9*	11.1	3.48
313 mg/kg	A	.	6	9	4	4	8	8	12	10	9	*9*	7.8	2.68
	B	.	.	13	14	10	10	7	7	8	6	*8*	9.4	2.92
	C	11	18	14	8	12	9	21	9	8	10	*10*	12.0	4.42
	D	.	8	15	7	16	7	10	7	8	6	*9*	9.3	3.67
1250 mg/kg	A	.	6	6	11	7	7	7	9	6	10	*9*	7.7	1.87
	B	.	.	.	.	7	5	6	6	4	5	*6*	5.5	1.05
	C	6	4	8	12	8	8	4	8	11	11	*10*	8.0	2.79
	D	8	8	7	9	9	8	5	6	11	6	*10*	7.7	1.77
5000 mg/kg	A	9	6	8	7	9	7	8	9	6	11	*10*	8.0	1.56
	B	.	.	10	8	6	5	15	12	7	6	*8*	8.6	3.46
	C	.	.	9	5	5	9	6	7	8	7	*8*	7.0	1.60
	D	5	6	6	7	7	7	8	.	.	.	*7*	6.6	0.98

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

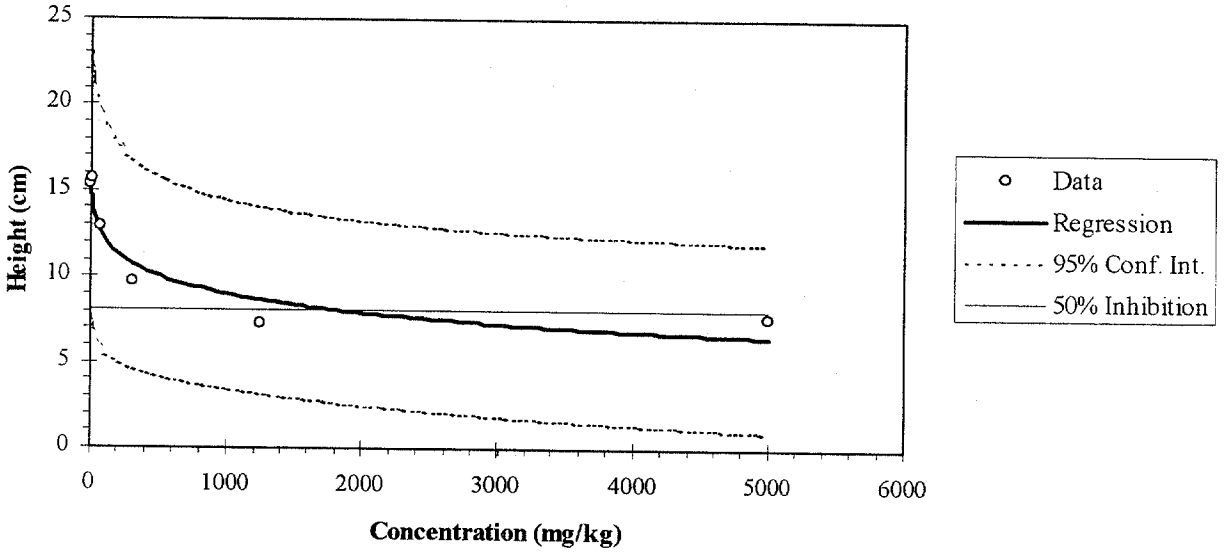
Appendix 9.8

Ryegrass Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	14.2	15.5	17.1	14.6	*4*	15.4	1.29
20 mg/kg	17.3	17.1	14.8	13.5	*4*	15.7	1.85
78 mg/kg	12.4	13.4	14.8	11.1	*4*	13.0	1.56
313 mg/kg	7.8	9.4	12.0	9.3	*4*	9.6	1.75
1250 mg/kg	7.7	5.5	8.0	7.7	*4*	7.2	1.15
5000 mg/kg	8.0	8.6	7.0	6.6	*4*	7.5	0.93

Appendix 9.9

Mean Ryegrass Height on Day 21



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
113.815	0.87116	14869.62	16.1050	1.7781	0.86700

EC_{50}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
1801.36	110.688	29315.68	16.1050	1.7781	0.86700

Appendix 9.10

Ryegrass Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
20 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
78 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
313 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
1250 mg/kg	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	.	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	*6*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
5000 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	.	.	*7*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

Appendix 10.1

Soybean Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	9	10	9	*4*	9.50	0.58
20 mg/kg	10	8	9	9	*4*	9.00	0.82
78 mg/kg	9	9	10	9	*4*	9.25	0.50
313 mg/kg	10	9	9	10	*4*	9.50	0.58
1250 mg/kg	10	10	9	9	*4*	9.50	0.58
5000 mg/kg	9	9	9	9	*4*	9.00	0.00

Day 14

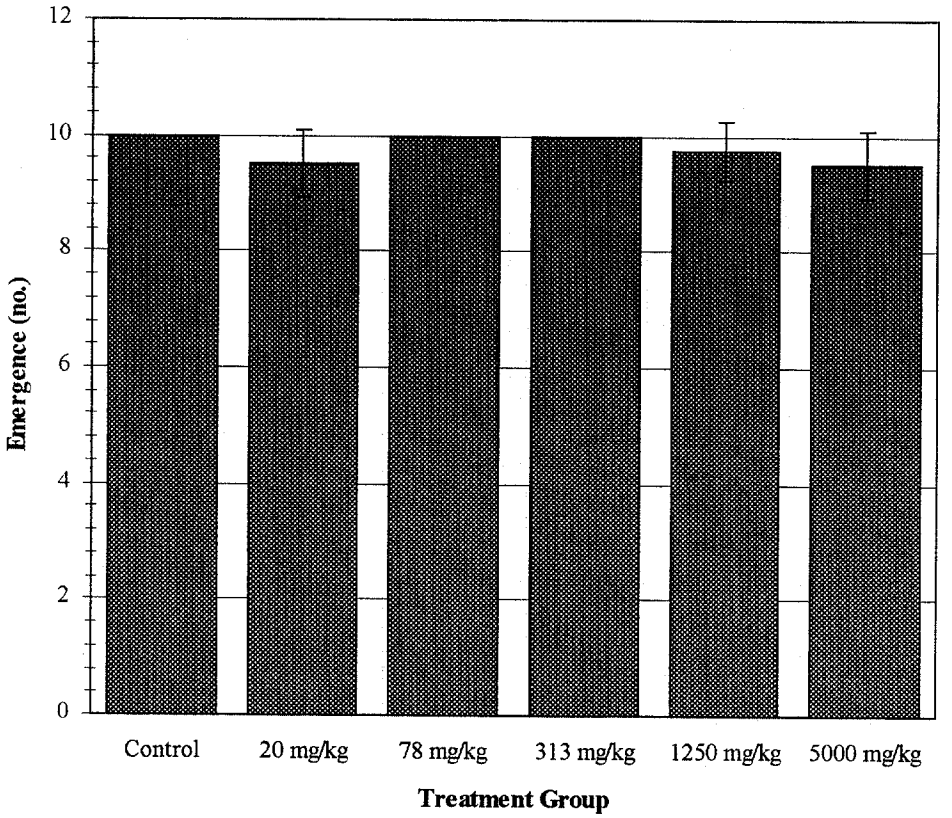
Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
20 mg/kg	10	9	9	10	*4*	9.50	0.58
78 mg/kg	10	10	10	10	*4*	10.00	0.00
313 mg/kg	10	10	10	10	*4*	10.00	0.00
1250 mg/kg	10	10	9	10	*4*	9.75	0.50
5000 mg/kg	9	9	10	9	*4*	9.25	0.50

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
20 mg/kg	10	9	9	10	*4*	9.50	0.58
78 mg/kg	10	10	10	10	*4*	10.00	0.00
313 mg/kg	10	10	10	10	*4*	10.00	0.00
1250 mg/kg	10	10	9	10	*4*	9.75	0.50
5000 mg/kg	10	9	10	9	*4*	9.50	0.58

Appendix 10.2

Mean Soybean Emergence on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p > 0.05$).

Appendix 10.3

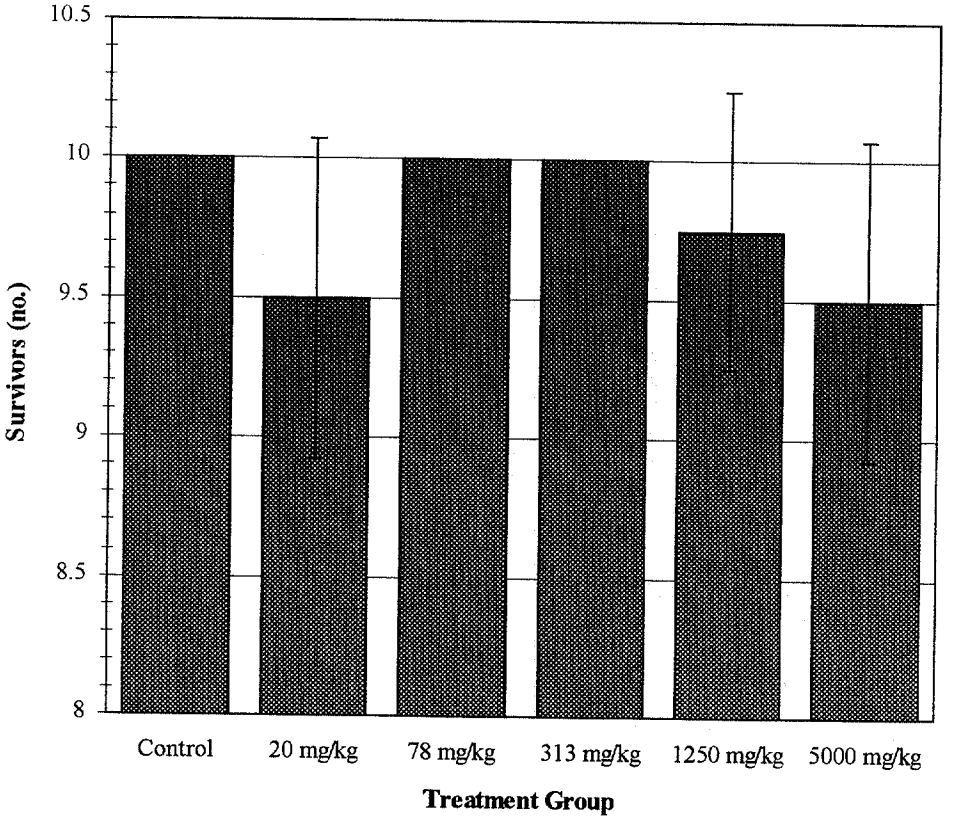
Soybean 21-Day Survival

Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	10	10	10	10	*4*	10.00	0.00
20 mg/kg	10	9	9	10	*4*	9.50	0.58
78 mg/kg	10	10	10	10	*4*	10.00	0.00
313 mg/kg	10	10	10	10	*4*	10.00	0.00
1250 mg/kg	10	10	9	10	*4*	9.75	0.50
5000 mg/kg	10	9	10	9	*4*	9.50	0.58

Appendix 10.4

Mean Soybean 21-Day Survival



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

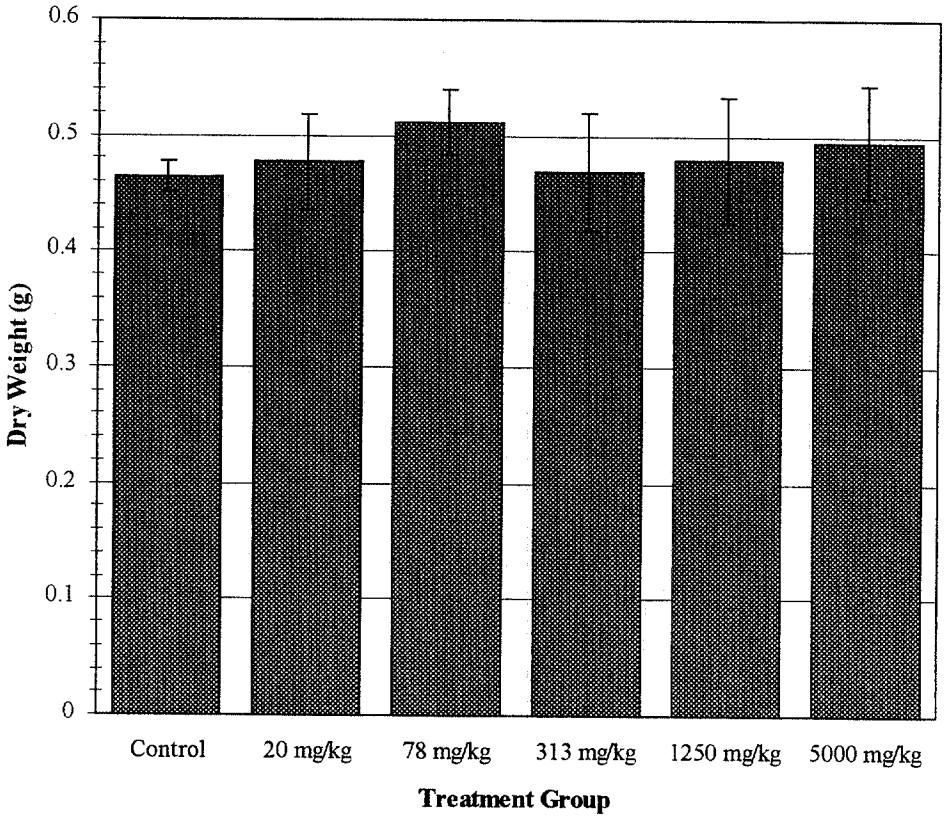
Appendix 10.5

Soybean Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.48	0.47	0.46	0.45	*4*	0.46	0.014
20 mg/kg	0.47	0.54	0.44	0.46	*4*	0.48	0.041
78 mg/kg	0.55	0.49	0.51	0.49	*4*	0.51	0.028
313 mg/kg	0.51	0.50	0.40	0.47	*4*	0.47	0.051
1250 mg/kg	0.52	0.43	0.53	0.43	*4*	0.48	0.054
5000 mg/kg	0.45	0.50	0.47	0.56	*4*	0.49	0.049

Appendix 10.6

Mean Soybean Dry Weight



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

Appendix 10.7

Soybean Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	27	19	24	20	23	26	19	27	20	25	*10*	23.0	3.27
	B	30	20	26	25	21	24	27	26	23	21	*10*	24.3	3.13
	C	21	22	21	20	21	19	21	25	23	21	*10*	21.4	1.65
	D	20	23	22	22	24	18	19	20	10	18	*10*	19.6	3.95
20 mg/kg	A	23	26	29	29	15	24	24	24	27	25	*10*	24.6	3.98
	B	.	20	21	28	21	24	25	23	25	23	*9*	23.3	2.50
	C	.	22	25	25	26	24	17	23	17	16	*9*	21.7	3.94
	D	19	19	22	24	20	21	22	19	25	20	*10*	21.1	2.13
78 mg/kg	A	18	25	32	28	21	22	25	27	24	21	*10*	24.3	4.06
	B	21	24	23	20	20	24	25	24	23	16	*10*	22.0	2.75
	C	26	26	22	28	24	18	25	17	27	21	*10*	23.4	3.78
	D	11	22	21	25	23	20	18	17	24	14	*10*	19.5	4.50
313 mg/kg	A	5	19	26	26	27	24	21	19	27	17	*10*	21.1	6.76
	B	13	20	32	29	29	12	29	30	25	24	*10*	24.3	7.12
	C	10	21	23	18	17	11	16	28	24	22	*10*	19.0	5.72
	D	26	25	21	27	22	26	25	26	27	25	*10*	25.0	2.00
1250 mg/kg	A	22	26	27	26	18	25	11	28	24	23	*10*	23.0	5.10
	B	21	21	23	23	24	28	21	23	24	17	*10*	22.5	2.84
	C	.	27	23	27	29	23	28	27	19	26	*9*	25.4	3.17
	D	15	24	23	21	19	23	18	24	16	20	*10*	20.3	3.27
5000 mg/kg	A	19	21	22	31	5	27	30	25	28	3	*10*	21.1	9.81
	B	.	20	19	14	23	24	3	18	23	24	*9*	18.7	6.75
	C	20	24	21	25	25	17	19	21	15	25	*10*	21.2	3.55
	D	.	21	21	24	20	27	19	27	24	26	*9*	23.2	3.07

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

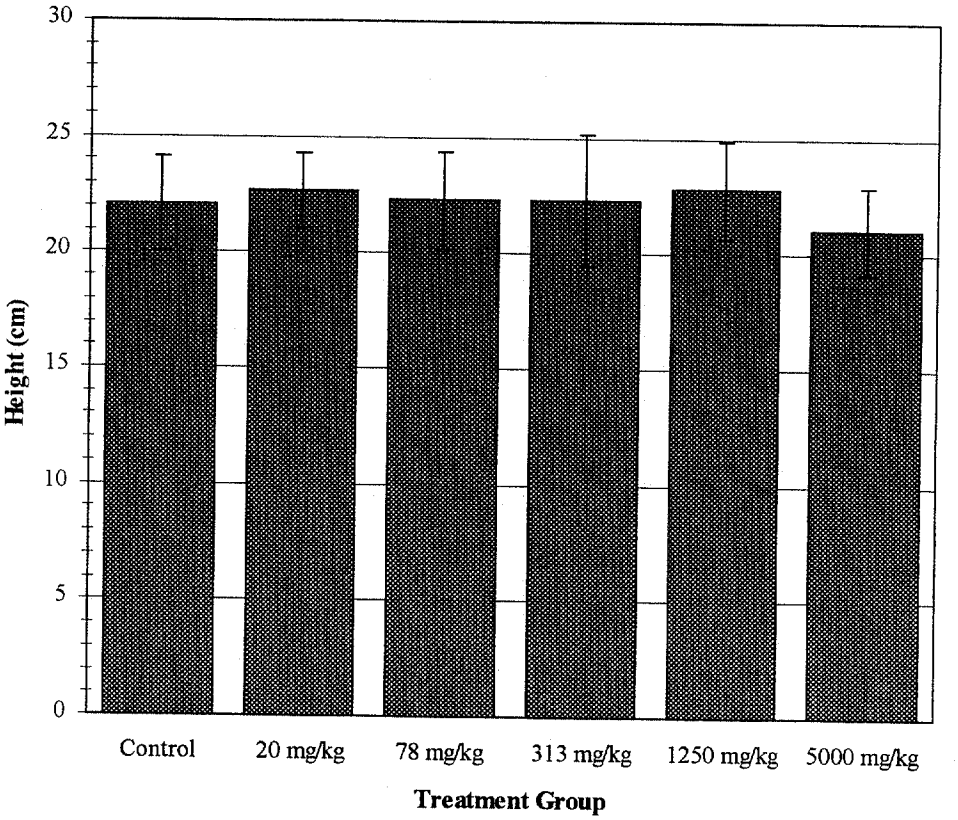
Appendix 10.8

Soybean Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	23.0	24.3	21.4	19.6	*4*	22.1	2.03
20 mg/kg	24.6	23.3	21.7	21.1	*4*	22.7	1.60
78 mg/kg	24.3	22.0	23.4	19.5	*4*	22.3	2.09
313 mg/kg	21.1	24.3	19.0	25.0	*4*	22.4	2.81
1250 mg/kg	23.0	22.5	25.4	20.3	*4*	22.8	2.11
5000 mg/kg	21.1	18.7	21.2	23.2	*4*	21.0	1.86

Appendix 10.9

Mean Soybean Height on Day 21



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

Appendix 10.10

Soybean Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
20 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
78 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	20.LC	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	30.LC	*10*	5	10.8
313 mg/kg	A	60.LC	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	6	19.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
1250 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
5000 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	70.LC	0.-	10.SC	0.-	*9*	9	23.2
	C	0.-	0.-	0.-	0.-	0.-	30.LC	0.-	0.-	0.-	0.-	*10*	3	9.5
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0

[1]The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity. LC – Leaf Curl, SC – Stem Curl

Appendix 11.1

Tomato Emergence

Day 7

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	7	3	7	3	*4*	5.00	2.31
20 mg/kg	8	10	8	5	*4*	7.75	2.06
78 mg/kg	5	6	5	3	*4*	4.75	1.26
313 mg/kg	8	8	9	5	*4*	7.50	1.73
1250 mg/kg	1	8	5	5	*4*	4.75	2.87
5000 mg/kg	7	8	6	4	*4*	6.25	1.71

Day 14

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	10	9	*4*	9.50	0.58
20 mg/kg	8	10	8	9	*4*	8.75	0.96
78 mg/kg	10	9	8	9	*4*	9.00	0.82
313 mg/kg	9	9	9	9	*4*	9.00	0.00
1250 mg/kg	7	10	10	10	*4*	9.25	1.50
5000 mg/kg	9	10	8	9	*4*	9.00	0.82

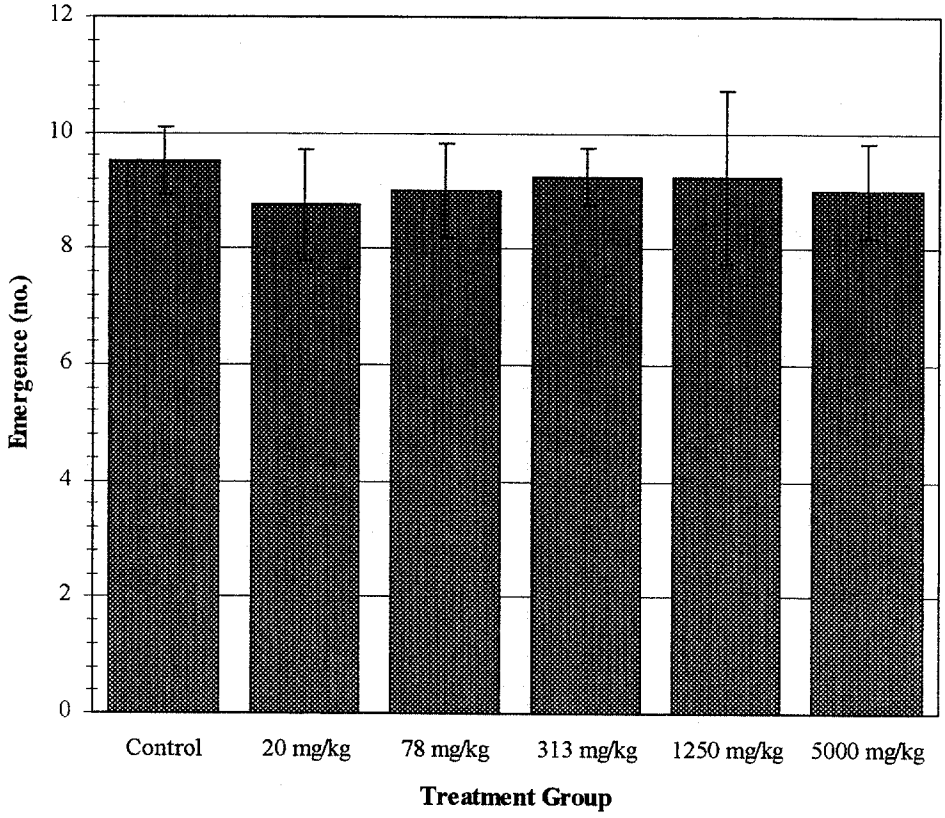
Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	10	9	*4*	9.50	0.58
20 mg/kg	8	10	8	9	*4*	8.75	0.96
78 mg/kg	10	9	8	9	*4*	9.00	0.82
313 mg/kg	10	9	9	9	*4*	9.25	0.50
1250 mg/kg	7	10	10[1]	10	*4*	9.25	1.50
5000 mg/kg	9	10	8	9	*4*	9.00	0.82

[1] An eleventh seedling was observed in this replicate on Day 21. Mean emergence was based on 10 emerged seedlings per 10 planted seeds.

Appendix 11.2

Mean Tomato Emergence on Day 21



No treatment group mean is significantly different from the control mean (Dunnettt's test, $p>0.05$).

Appendix 11.3

Tomato 21-Day Survival

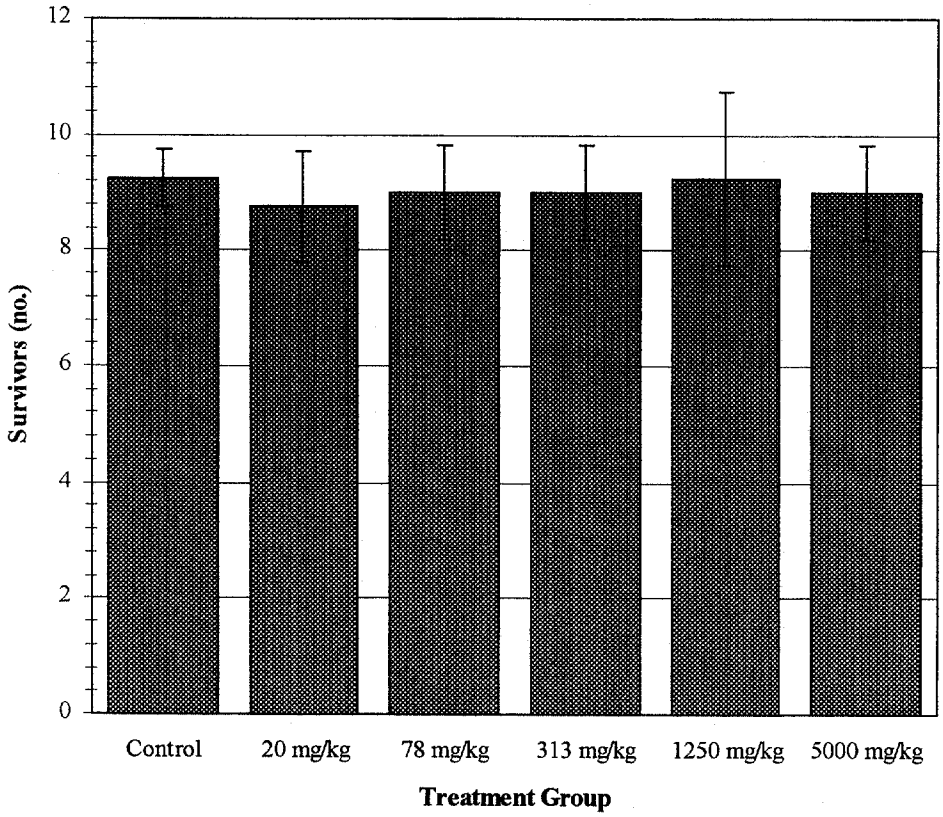
Day 21

Treatment Group	Number of Emerged Seedlings in Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	9	10	9	9	*4*	9.25	0.50
20 mg/kg	8	10	8	9	*4*	8.75	0.96
78 mg/kg	10	9	8	9	*4*	9.00	0.82
313 mg/kg	10	8	9	9	*4*	9.00	0.82
1250 mg/kg	7	10	10[1]	10	*4*	9.25	1.50
5000 mg/kg	9	10	8	9	*4*	9.00	0.82

[1] An eleventh seedling was observed in this replicate on Day 21. Mean survival was based on 10 surviving seedlings per 10 planted seeds.

Appendix 11.4

Mean Tomato 21-Day Survival



No treatment group mean is significantly different from the control mean (Dunnett's test, $p>0.05$).

Appendix 11.5

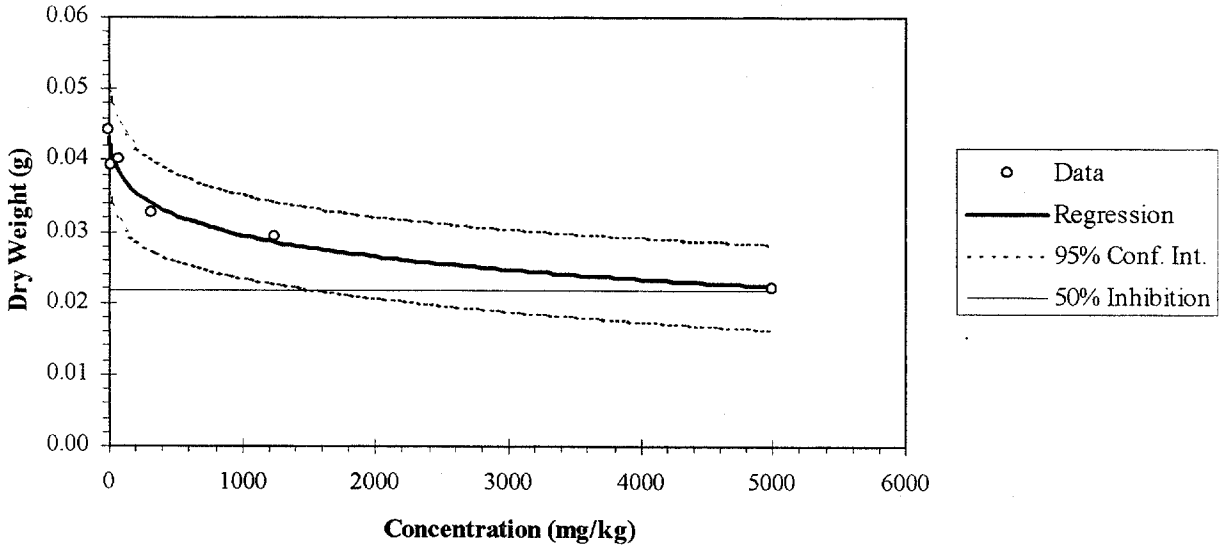
Tomato Mean Seedling Dry Weight, Day 21

Treatment Group	Mean Weight (g) per Plant of Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	0.034	0.048	0.040	0.054	*4*	0.044	0.0088
20 mg/kg	0.039	0.035	0.055	0.028	*4*	0.039	0.0115
78 mg/kg	0.049	0.046	0.036	0.030	*4*	0.040	0.0087
313 mg/kg	0.041	0.025	0.032	0.032	*4*	0.033	0.0065
1250 mg/kg	0.024	0.037	0.027[1]	0.029	*4*	0.029	0.0055
5000 mg/kg	0.020	0.019	0.024	0.026	*4*	0.022	0.0031

[1] Eleven plants were observed in replicate. Weight was not adjusted for consideration of eleventh plant weighed.

Appendix 11.6

Mean Tomato Dry Weight



Curve Parameters

EC_{25}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
422.182	81.1895	2195.84	0.0438	1.6476	0.97168

EC_{50}	Lower 95% Confidence Limit	Upper 95% Confidence Limit	R_0	σ	r^2
5455.07	1962.00	15167.01	0.0438	1.6476	0.97168

Appendix 11.7

Tomato Seedling Height on Day 21

Treatment Group	Replicate	Height (cm) for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	4	7	3	5	5	5	6	7	4	*9*	5.1	1.36
	B	4	6	3	5	5	11	7	5	6	7	*10*	5.9	2.18
	C	.	5	6	5	5	5	6	6	5	6	*9*	5.4	0.53
	D	.	6	7	7	7	6	3	7	4	6	*9*	5.9	1.45
20 mg/kg	A	.	.	7	7	5	7	5	6	4	5	*8*	5.8	1.16
	B	3	4	4	4	5	6	6	5	5	4	*10*	4.6	0.97
	C	.	.	4	7	3	7	8	6	4	6	*8*	5.6	1.77
	D	.	4	3	3	5	5	5	4	4	3	*9*	4.0	0.87
78 mg/kg	A	4	5	5	6	5	6	6	8	6	4	*10*	5.5	1.18
	B	.	4	5	4	5	7	7	7	5	7	*9*	5.7	1.32
	C	4	3	4	6	5	4	5	6	.	.	*8*	4.6	1.06
	D	.	3	4	4	2	5	4	4	5	6	*9*	4.1	1.17
313 mg/kg	A	4	6	6	6	6	5	4	5	3	6	*10*	5.1	1.10
	B	.	.	4	5	5	4	4	3	5	7	*8*	4.6	1.19
	C	.	4	2	5	4	4	5	5	6	8	*9*	4.8	1.64
	D	.	6	7	4	3	4	4	4	4	8	*9*	4.9	1.69
1250 mg/kg	A	.	.	.	3	4	3	3	3	5	4	*7*	3.6	0.79
	B	4	6	4	3	3	5	6	6	7	4	*10*	4.8	1.40
	C[1]	5	5	5	4	6	4	4	4	5	4	*10*	4.6	0.70
	D	3	5	6	5	5	5	5	6	2	4	*10*	4.6	1.26
5000 mg/kg	A	3	4	7	5	4	4	5	5	5	.	*9*	4.7	1.12
	B	4	3	4	3	4	5	3	4	6	7	*10*	4.3	1.34
	C	.	.	4	4	5	5	4	3	4	8	*8*	4.6	1.51
	D	.	4	5	5	5	7	5	4	4	4	*9*	4.8	0.97

The "." symbol indicates that the seedling either did not emerge or died prior to measurement.

[1] An eleventh seedling 3 cm tall was observed in this replicate, but was dropped from calculation of the replicate mean.

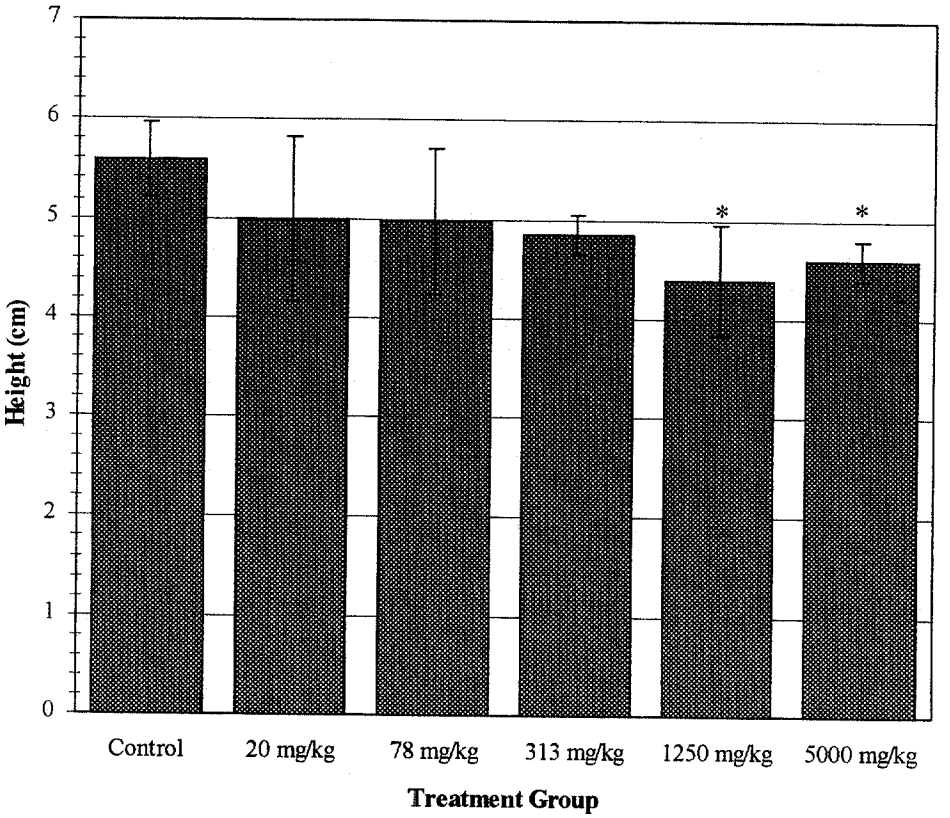
Appendix 11.8

Tomato Mean Seedling Height on Day 21

Treatment Group	Mean Height (cm) for Replicate: A	B	C	D	*n*	Mean	Std. Dev.
Control	5.1	5.9	5.4	5.9	*4*	5.6	0.38
20 mg/kg	5.8	4.6	5.6	4.0	*4*	5.0	0.84
78 mg/kg	5.5	5.7	4.6	4.1	*4*	5.0	0.74
313 mg/kg	5.1	4.6	4.8	4.9	*4*	4.8	0.20
1250 mg/kg	3.6	4.8	4.6	4.6	*4*	4.4	0.56
5000 mg/kg	4.7	4.3	4.6	4.8	*4*	4.6	0.21

Appendix 11.9

Mean Tomato Height on Day 21



* Treatment group mean is significantly different from the control mean (Dunnett's test, $p<0.05$).

Appendix 11.10

Tomato Seedling Condition, Day 21

Treatment Group	Replicate	Condition (score.sign)[1] for Plant Number:										*n*	Mean	Std. Dev.
		1	2	3	4	5	6	7	8	9	10			
Control	A	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	10	31.6
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
20 mg/kg	A	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
78 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	C	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	.	*8*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
313 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	B	.	100.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	11	33.3
	C	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0
1250 mg/kg	A	.	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*7*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C[2]	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	D	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
5000 mg/kg	A	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	.	*9*	0	0.0
	B	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*10*	0	0.0
	C	.	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*8*	0	0.0
	D	.	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	0.-	*9*	0	0.0

[1] The "." symbol indicates that the seedling did not emerge. A score of 0 indicates a normal seedling, while a score of 100 indicates a dead seedling. Intermediate scores are assigned to indicate the relative severity of observed signs of toxicity.

[2] An eleventh apparently normal seedling was observed in this replicate.